

SEARCH REQUEST FORM

Requestor's Name: Garnette Draper Serial Number: 08/1785700
Date: 1-29-98 Phone: 301-463-2 Art Unit: 17

Search Topic:

Please write a detailed statement of search topic. Describe specifically as possible the subject matter to be searched. Define any terms that may have a special meaning. Give examples or relevant citations, authors, keywords, etc., if known. For sequences, please attach a copy of the sequence. You may include a copy of the broadest and/or most relevant claim(s).

Draper, Garnette

From: Draper, Garnette
Sent: Thursday, January 29, 1998 3:37 PM
To: Sheppard, Paula
Subject: word search
Importance: High

Hi, I just got this rather old date case and I was wondering how soon you could do this word search for me (maybe by COB Friday). I'm sure that Paula Hutzell or George Elliot would ok it if it is written.

1. (IL-4 or interleukin- 4 or any other synonymous names)
2. (mutant or mutein or derivative, or replac? or substitut? or modif? or agonist or antagonist)
3. (residues/amino acids at positions 121, 124 or 125) or [alternatively at residues 145, 148 or 149]

Not interested in above alone, but as crossed below: ALSO NO HITS AFTER 1995

- A. search 1 and 2 and 3
- B. search 1 and 2 and (N-termin?)
- C. search 1 and 2 and (C-termin?)
- D. search 1 and 2 and glycosylat?)

THANKS G

Ruth

STAFF USE ONLY

Date completed: 1-30-98
Searcher: Ruth
Terminal time: 5:30
Elapsed time: 1 hr 12 min
CPU time: ?
Total time: ?
Number of Searches: 1
Number of Databases: 13

Search Site	Vendors
<input type="checkbox"/> STIC	<input type="checkbox"/> IG
<input checked="" type="checkbox"/> CM-1	<input type="checkbox"/> STN
<input type="checkbox"/> Pre-S	<input type="checkbox"/> Dialog
Type of Search	
<input type="checkbox"/> N.A. Sequence	<input type="checkbox"/> APS
<input type="checkbox"/> A.A. Sequence	<input type="checkbox"/> Geninfo
<input type="checkbox"/> Structure	<input type="checkbox"/> SDC
<input checked="" type="checkbox"/> Bibliographic	<input type="checkbox"/> DARC/Questel
	<input type="checkbox"/> Other

=> fil wpids; d que 144; fil capl; d que 120

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FILE LAST UPDATED: 26 JAN 1998 <19980126/UP>

>>>UPDATE WEEKS:

MOST RECENT DERWENT WEEK 199804 <199804/DW>

DERWENT WEEK FOR CHEMICAL CODING: 199750

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L40 185 SEA FILE=WPIDS ABB=ON (INTERLEUKIN OR IL) (W) 4 OR IL4
L41 73 SEA FILE=WPIDS ABB=ON L40 NOT PY>1995
L42 549 SEA FILE=WPIDS ABB=ON (POSITION# OR AMINO ACID# OR RESID
UE#) (5A) (121 OR 124 OR 125 OR 145 OR 148 OR 149)
L43 78 SEA FILE=WPIDS ABB=ON L40(L) (MUTANT? OR MUTAT? OR MUTEIN
? OR DERIV? OR REPLAC? OR SUBSTITUT? OR MODIF? OR AGONIST
? OR ANTAGONIST?)
L44 0 SEA FILE=WPIDS ABB=ON L43 AND L42 AND L41

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FILE COVERS 1967 - 30 Jan 1998 VOL 128 ISS 5
FILE LAST UPDATED: 30 Jan 1998 (980130/ED)

This file contains CAS Registry Numbers for easy and accurate substance identification.

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L5 40 SEA FILE=REGISTRY ABB=ON INTERLEUKIN 4?/CN
L6 8612 SEA FILE=CAPLUS ABB=ON L5 OR (INTERLEUKIN OR IL) (W) 4
L7 8689 SEA FILE=CAPLUS ABB=ON L6 OR IL4
L8 211758 SEA FILE=CAPLUS ABB=ON MUTANT? OR MUTAT? OR MUTEIN?
L9 508178 SEA FILE=CAPLUS ABB=ON DERIVATIVE#
L10 1066629 SEA FILE=CAPLUS ABB=ON REPLAC? OR SUBSTITUT? OR MODIF?
L11 168570 SEA FILE=CAPLUS ABB=ON AGONIST? OR ANTAGONIST?
L12 1097 SEA FILE=CAPLUS ABB=ON L7(L) ((L8 OR L9 OR L10 OR L11))
L14 2993 SEA FILE=CAPLUS ABB=ON (POSITION# OR AMINO ACID# OR RESI
DUE#) (5A) (121 OR 124 OR 125 OR 145 OR 148 OR 149)
L15 9 SEA FILE=CAPLUS ABB=ON L12 AND L14
L20 7 SEA FILE=CAPLUS ABB=ON L15 NOT PY>1995

=> d bib ab 120 1-7

L20 ANSWER 1 OF 7 CAPLUS COPYRIGHT 1998 ACS
AN 1994:628408 CAPLUS
DN 121:228408
TI Design of human **interleukin-4**
antagonists inhibiting **interleukin-4**
-dependent and interleukin-13-dependent responses in T-cells and
B-cells with high efficiency
AU Tony, Hans-Peter; Shen, Bo-Jiang; Reusch, Petra; Sebald, Walter
CS Medizinische Poliklinik, Univ. Wuerzburg, Wuerzburg, D-97074,
Germany
SO Eur. J. Biochem. (1994), 225(2), 659-65
CODEN: EJBCAI; ISSN: 0014-2956
DT Journal
LA English
AB Human **interleukin-4** possesses two distinct sites
for receptor activation. A signalling site, comprising residues
near the C-terminus on helix D, dets. the efficacy of
interleukin-4 signal transduction without
affecting the binding to the **interleukin-4**
receptor .alpha. subunit. A complete **antagonist** and a
series of low-efficacy **agonist** variants of human
interleukin-4 could be generated by introducing
combinations of two or three neg. charged aspartic acid
residues in this site at **positions 121,**
124, and **125.** One of the double variants,
designated **[R121D, Y124D]interleukin-4**, with
replacements of both Arg121 and Tyr124 by aspartic acid
residues was completely inactive in all analyzed cellular responses.
The loss of efficacy in **[R121D, Y124D]interleukin-**
4 is estd. to be larger than 2000-fold. Variant **[R121D,**
Y124D]interleukin-4 was also a perfect
antagonist for inhibition of interleukin-13-dependent
responses in B-cells and the TF-1 cell line with a Ki value of
approx. 100 pM. In addn., inhibition of both **interleukin-**
4-induced and IL-13-induced responses could be obtained by
monoclonal antibody X2/45 raised against interleukin-4Rex, the
extracellular domain of the **interleukin-4**
receptor .alpha. subunit. These results indicate that efficient
interleukin-4 antagonists can be
designed on the basis of a sequential two-step activation model. In
addn., the expts. indicate the functional participation of the
interleukin-4 receptor .alpha. subunit in the
interleukin-13 receptor system.

L20 ANSWER 2 OF 7 CAPLUS COPYRIGHT 1998 ACS
AN 1994:531810 CAPLUS
DN 121:131810
TI Aspects of receptor binding and signaling of interleukin-4
investigated by site-directed mutagenesis and NMR spectroscopy
AU Mueller, Thomas; Dieckmann, Thorsten; Sebald, Walter; Oschkinat,
Hartmut
CS Theodor-Boveri-Inst., Univ. Wuerzburg, Wuerzburg, D-97074, Germany
SO J. Mol. Biol. (1994), 237(4), 423-36
CODEN: JMOPAK; ISSN: 0022-2836
DT Journal
LA English
AB Cytokines are hormones that carry information from cell to cell.
This information is read from their surface upon binding to
transmembrane receptors and by the subsequent initiation of receptor
oligomerization. An influence on this process through mutagenesis
on the hormone surface is highly desirable for medical reasons.
Searched by Barb O'Bryen, STIC 308-4291

However, an understanding of hormone-receptor interactions requires insight into the structural changes introduced by the **mutations**. In this line structural studies on human **IL-4** and the medically important **IL-4 antagonists Y124D and Y124G** are presented. The site around Y124 is an important epitope responsible for the ability of **IL-4** to cause a signal in the target cells. It is shown that the local main-chain structure around **residue 124** in the variants remains unchanged. A strategy is presented here which allows the study of these types of proteins and their variants by NMR which does not require carbon labeled samples.

L20 ANSWER 3 OF 7 CAPLUS COPYRIGHT 1998 ACS
AN 1994:52393 CAPLUS
DN 120:52393
TI Two distinct functional sites of human interleukin 4 are identified by variants impaired in either receptor binding or receptor activation
AU Kruse, N.; Shen, B. J.; Arnold, S.; Tony, H. P.; Mueller, T.; Sebald, W.
CS Theodor-Boveri-Inst. Biowiss., Univ. Wuerzburg, Wuerzburg, D-97074, Germany
SO EMBO J. (1993), 12(13), 5121-9
CODEN: EMJODG; ISSN: 0261-4189
DT Journal
LA English
AB **Interleukin 4 (IL-4)** exerts a decisive role in the coordination of protective immune responses against parasites, particularly helminths. A disregulation of **IL-4** function is possibly involved in the genesis of allergic disease states. The search for important amino acid residues in human **IL-4** by **mutational anal.** of charged invariant amino acid positions identified two distinct functional sites in the 4-helix-bundle protein. Site 1 was marked by amino acid **substitutions** of the glutamic acid at position 9 in helix A and arginine at position 88 in helix C. Exchanges at both positions led to **IL-4** variants deficient in binding to the extracellular domain of the **IL-4** receptor (**IL-4Rex**). In parallel, ≥ 1000 -fold increased concns. of this type of variant were required to induce T-cell proliferation and B-cell CD23 expression. Site 2 was marked by amino acid exchanges in helix D at **positions 121, 124, and 125** (arginine, tyrosine, and serine resp. in the wild-type). **IL-4** variants affected at site 2 exhibited partial **agonist** activity during T-cell proliferation; however, they still bound with high affinity to **IL-4Rex**. The generation of an **IL-4 antagonist** by replacing tyrosine 124 with aspartic acid has been described before by Kruse et al. (1992). Thus, **IL-4** functions by binding **IL-4Rex** via site 1 which is constituted by residues on helices A and C. Also, the assocn. of a second, still undefined receptor protein within site 2 in helix D activates the receptor system and generates a transmembrane signal.

L20 ANSWER 4 OF 7 CAPLUS COPYRIGHT 1998 ACS
AN 1993:623986 CAPLUS
DN 119:223986
TI An **interleukin 4 (IL-4)** **mutant** protein inhibits both **IL-4** or **IL-13**-induced human immunoglobulin G4 (IgG4) and IgE synthesis and B Searched by Barb O'Bryen, STIC 308-4291

cell proliferation: Support for a common component shared by
IL-4 and IL-13 receptors

AU Versa, Gregorio; Punnonen, Juha; Cocks, Benjamin G.; de Waal Malefyt, Rene; Vega, Felix, Jr.; Zurawski, Sandra M.; Zurawski, Gerard; de Vries, Jan E.

CS Hum. Immunol. Dep., DNAX Res. Inst., Palo Alto, CA, 94304-1104, USA

SO J. Exp. Med. (1993), 178(6), 2213-18

CODEN: JEMEAV; ISSN: 0022-1007

DT Journal

LA English

AB **Interleukin 4 (IL-4)** and IL-13 share many biol. functions. Both cytokines promote growth of activated human B cells and induce naive human sol. IgD+ (sIgD+) B cells to produce IgG4 and IgE. Here, a **mutant** form of human **IL-4**, in which the tyrosine residue at position 124 is replaced by aspartic acid (hIL-4.Y124D), specifically blocks IL-4 and IL-13-induced proliferation of B cells costimulated by anti-CD40 mAbs in a dose-dependent fashion. A mouse **mutant IL-4** protein (mIL-4.Y119D), which antagonizes the biol. activity of mouse **IL-4**, was ineffective. In addn., hIL-4.Y124D did not affect IL-2-induced B cell proliferation, demonstrating the specificity of the suppressive effect. HIL-4.Y124D did not have detectable **agonistic** activity in these B cell proliferation assays. Interestingly, hIL-4.Y124D also strongly inhibited both **IL-4** or IL-13-induced IgG4 and IgE synthesis in cultures of peripheral blood mononuclear cells, or high purified sIgD+ B cells cultured in the presence of anti-CD40 mAbs. **IL-4** and IL-13-induced IgE responses were inhibited >95% at a .apprx.50- or .apprx.20-fold excess of hIL-4.Y124D, resp., despite the fact that the **IL-4 mutant** protein had a weak **agonistic** activity. This **agonistic** activity was 1.6% of the maximal IgE responses induced by satg. concns. of **IL-4**. Taken together, these data indicate that there are commonalities between the **IL-4** and IL-13 receptor. In addn., since hIL-4.Y124D inhibited both **IL-4** and IL-13-induced IgE synthesis, it is likely that **antagonistic mutant IL-4** proteins may have potential clin. use in the treatment of IgE-mediated allergic disease.

L20 ANSWER 5 OF 7 CAPLUS COPYRIGHT 1998 ACS

AN 1993:470373 CAPLUS

DN 119:70373

TI Human **interleukin 4 (hIL-4) mutant** proteins as **antagonists** or partial **agonists** of hIL-4, their preparation and therapeutic use

IN Sebald, W.

PA Germany

SO Ger. Offen., 4 pp.

CODEN: GWXXBX

PI DE 4137333 A1 930519

AI DE 91-4137333 911113

DT Patent

LA German

AB Certain hIL-4 analogs, in which .gtoreq.1 of **amino acids** 120, 121, 123, **124, 125, 127**, or 128 is substituted with another natural amino acid, are prep'd. by recombinant DNA methodol. for use as hIL-4 competitive antagonists or partial agonists for treatment of allergy. Thus, cDNA for hIL-4 was subjected to site-directed mutagenesis with a Searched by Barb O'Bryen, STIC 308-4291

synthetic hexanucleotide to substitute Tyr124 with Gly and cloned in Escherichia coli JM103. The mutein had the same hIL-4 receptor-binding activity as the wild-type protein, but only 10-20% of the activity in inducing proliferation of activated peripheral blood cells.

L20 ANSWER 6 OF 7 CAPLUS COPYRIGHT 1998 ACS
AN 1992:569241 CAPLUS
DN 117:169241
TI Conversion of human **interleukin-4** into a high affinity **antagonist** by a single amino acid replacement
AU Kruse, N.; Tony, H. P.; Sebald, W.
CS Theodor-Boveri-Inst. Biowiss., Univ. Wuerzburg, Wuerzburg, D-8700, Germany
SO EMBO J. (1992), 11(9), 3237-44
CODEN: EMJODG; ISSN: 0261-4189
DT Journal
LA English
AB **Interleukin-4 (IL-4)**
represents a prototypic lymphokine. It promotes differentiation of B-cells and the proliferation of T- and B-cells, and other cell types of the lymphoid system. An **antagonist** of human IL-4 was discovered during the studies presented here after Tyr124 of the recombinant protein had been substituted by an aspartic acid residue. This IL-4 variant, Y124D, bound with high affinity to the IL-4 receptor ($K_D = 310 \text{ pM}$), but retained no detectable proliferative activity for T-cells and inhibited IL-4-dependent T-cell proliferation competitively ($K_i = 620 \text{ pM}$). The loss of efficacy in variant Y124D was estd. to be >100-fold on the basis of a weak partial **agonist** activity for the very sensitive induction of CD23 pos. B-cells. The substitution of Tyr124 by either phenylalanine, histidine, asparagine, lysine or glycine resulted in partial **agonist** variants with unaltered receptor binding affinity and relatively small deficiencies in efficacy. Thus, high affinity binding and signal generation can be uncoupled efficiently in a ligand of a receptor belonging to the recently identified hematopoietin receptor family. In addn. it is shown for the first time, that a powerful **antagonist** acting on the IL-4 receptor system can be derived from the IL-4 protein.

L20 ANSWER 7 OF 7 CAPLUS COPYRIGHT 1998 ACS
AN 1991:512499 CAPLUS
DN 115:112499
TI Site-directed mutagenesis reveals the importance of disulfide bridges and aromatic residues for structure and proliferative activity of human interleukin-4
AU Kruse, Niels; Lehrnbecher, Thomas; Sebald, Walter
CS Physiol.-Chem. Inst., Univ. Wuerzburg, Wuerzburg, D-8700, Fed. Rep. Ger.
SO FEBS Lett. (1991), 286(1-2), 58-60
CODEN: FEBLAL; ISSN: 0014-5793
DT Journal
LA English
AB **Mutant proteins (muteins)** of human **Interleukin-4 (IL4)** were constructed by means of in vitro mutagenesis. The **muteins** were expressed in Escherichia coli, submitted to a renaturation and purifn. protocol and analyzed for biol. activity. Exchange of the cysteines at either position 46 or 99 which form one of the three disulfide Searched by Barb O'Bryen, STIC 308-4291

bridges resulted in a nearly complete loss of biol. activity and an unstable protein. The exchange of tyrosine 124 also inactivated the protein, while a **mutation** of tyrosine 56 left some residual activity. Exchange of the other four cysteines or of the single tryptophan had smaller effects.

=> fil capl; d que 136; s 136 not 120; fil wpid; d que 148

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L7	8689	SEA FILE=CAPLUS ABB=ON	L6 OR IL4
L8	211758	SEA FILE=CAPLUS ABB=ON	MUTANT? OR MUTAT? OR MUTEIN?
L9	508178	SEA FILE=CAPLUS ABB=ON	DERIVATIVE#
L10	1066629	SEA FILE=CAPLUS ABB=ON	REPLAC? OR SUBSTITUT? OR MODIF?
L11	168570	SEA FILE=CAPLUS ABB=ON	AGONIST? OR ANTAGONIST?
L12	1097	SEA FILE=CAPLUS ABB=ON	L7(L)((L8 OR L9 OR L10 OR L11))
L17	288781	SEA FILE=CAPLUS ABB=ON	TERMIN?
L18	79959	SEA FILE=CAPLUS ABB=ON	(N OR AMIN?) (2A)L17
L35	12	SEA FILE=CAPLUS ABB=ON	L18(S)((L8 OR L9 OR L10 OR L11))
		AND L12	
L36	7	SEA FILE=CAPLUS ABB=ON	L35 NOT PY>1995

L52 7 L36 NOT L20 previously printed

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L40 185 SEA FILE=WPIDS ABB=ON (INTERLEUKIN OR IL) (W) 4 OR IL4
L41 73 SEA FILE=WPIDS ABB=ON L40 NOT PY>1995
L43 78 SEA FILE=WPIDS ABB=ON L40(L) (MUTANT? OR MUTAT? OR MUTEIN?
? OR DERIV? OR REPLAC? OR SUBSTITUT? OR MODIF? OR AGONIST?
? OR ANTAGONIST?)
L45 314299 SEA FILE=WPIDS ABB=ON TERMIN?
Searched by Barb O'Bryen, STIC 308-4291

L46 6455 SEA FILE=WPIDS ABB=ON (N OR AMIN?) (2A)L45
L48 2 SEA FILE=WPIDS ABB=ON L43 AND L46 AND L41

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=> d.bib ab 153 1-9

L53 ANSWER 1 OF 9 CAPLUS COPYRIGHT 1998 ACS
AN 1995:996652 CAPLUS
DN 124:97712
TI Circularly permuted ligands and circularly permuted chimeric molecules
IN Pastan, Ira; Kreitman, Robert J.
PA United States Dept. of Health and Human Services, USA
SO PCT Int. Appl., 97 pp.
CODEN: PIXXD2
PI WO 9527732 A2 951019
DS W: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI,
GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LU, LV, MD,
MG, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ,
TM, TT
RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FR, GA, GB, GR,
IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG
AI WO 95-US4468 950406
PRAI US 94-225224 940408
DT Patent
LA English
AB This invention provides **modified** forms of ligands such as **interleukin 4 (IL4)** wherein the amino and carboxy ends are joined together, directly or through a linker, and new **amino** and carboxy **terminal** ends are formed at a different location within the ligand. These **modified** ligands are fully as active as the original ligands. Since the **modification** of the ligand represents a rearrangement of the mol., neither the function nor the desirability of such mols. was apparent prior to the work described here. Such rearranged mols. are referred to as circularly permuted mols. The present invention provides circularly permuted ligands which possess specificity and binding affinity comparable to or greater than the specificity and binding affinity of the original (unpermuted) ligand. The invention further provides for novel chimeric mols. comprising a circularly permuted ligand joined to one or more mols. of interest. These are suitable for targeted drug delivery to tumor cells.

L53 ANSWER 2 OF 9 CAPLUS COPYRIGHT 1998 ACS
AN 1995:722434 CAPLUS
DN 123:187867
TI Increased antitumor activity of a circularly permuted interleukin 4-toxin in mice with interleukin 4 receptor-bearing human carcinoma
Searched by Barb O'Bryen, STIC 308-4291

AU Kreitman, Robert J.; Puri, Raj K.; Pastan, Ira
CS Cent. Biol. Eval. Res., Food Drug Adm., Bethesda, MD, 20892, USA
SO Cancer Res. (1995), 55(15), 3357-63
CODEN: CNREA8; ISSN: 0008-5472
DT Journal
LA English
AB The authors reported previously that circularly permuted **interleukin-4 (IL4)**, composed of amino acids 38-129 of **IL4** connected by a linker peptide GGNNG to amino acids 1-37, is preferable to native **IL4** for fusing to the N terminus of truncated *Pseudomonas exotoxin* (PE) to make a recombinant toxin, because the new ligand-toxin junction results in improved **IL4** receptor (IL4R)-binding (R. J. Kreitman et al., 1994). The authors now report that the improved binding of circularly permuted **IL4**-toxin is assocd. with improved antitumor activity in tumor-bearing mice. For in vivo testing, the authors made an improved circularly permuted **IL4**-toxin, termed **IL4(38-37)-PE38KDEL**. It contains an N38D **mutation at the N terminus**, allowing improved expression and large-scale prodn. in *Escherichia coli*. It also contains the truncated toxin PE38KDEL, which is composed of amino acids 253-364 and 381-608 of PE, followed by KDEL. To evaluate antitumor activity, nude mice carrying s.c. tumors composed of IL4R-bearing human A431 epidermoid carcinoma cells were injected with recombinant toxins i.v. every other day for 3 doses. **IL4(38-37)-PE38KDEL** induced complete remission in 80% of mice receiving 50 .mu.g/kg .times. 3 and 100% of mice receiving 100 .mu.g/kg .times. 3, while only 70% of mice receiving 200 .mu.g/kg .times. 3 of the native **IL4**-toxin **IL4-PE38KDEL** obtained complete remission. Disease-free survival after obtaining complete remissions was higher in mice treated with **IL4(38-37)-PE38KDEL** 50 .mu.g/Kg QOD .times. 3 than with **IL4-PE38KDEL** 200 .mu.g/Kg QOD .times. 3. **IL4(38-37)-PE38KDEL** and **IL4-PE38KDEL** exhibited similar toxicity and pharmacokinetics in the mice, indicating that the improved antitumor activity of the circularly permuted **IL4**-toxin was due to its improved binding to the IL4R on the target cells.

L53 ANSWER 3 OF 9 CAPLUS COPYRIGHT 1998 ACS
AN 1995:617564 CAPLUS
DN 123:53915
TI Circularly permuted interleukin 4 retains proliferative and binding activity
AU Kreitman, Robert J.; Puri, Raj K.; McPhie, Peter; Pastan, Ira
CS Lab. Mol. Biol. Div. Cancer Biology, National Cancer Inst., Bethesda, MD, 20892-4255, USA
SO Cytokine (1995), 7(4), 311-18
CODEN: CYTIE9; ISSN: 1043-4666
DT Journal
LA English
AB In human **interleukin 4 (IL-4)**, the carboxyl and amino termini of the 129 amino acid hormone are close to each other and this region is believed to be important for binding to the **IL-4** receptor (IL-4r). The authors constructed plasmids encoding circularly permuted **IL-4 mutants** with the peptide Gly-Gly-Asn-Gly-Gly (GGNNG) joining the carboxyl to the **amino terminus** and with new **amino** and carboxyl **termini** elsewhere. **Mutant IL-4(38-37)** is composed of **IL-4** residues 38-129, GGNNG and 1-37. **Mutant IL-4(105-104)** is composed of **IL-4** residues 105-129, GGNNG and Searched by Barb O'Bryen, STIC 308-4291

1-104. **IL-4(38-37)** and **IL-4**

(105-104) were purified from *E. coli* to near homogeneity and retained 50-100% of the binding and proliferative activity of **IL-4**, and in addn. retained the ability to upregulate CD23 on Burkitt's lymphoma cells. CD studies indicated that the tertiary structure of both **IL-4(38-37)** and **IL-4(105-104)** were retained, with the former mol. most similar to native **IL-4**. The authors conclude that while both native termini of **IL-4** may be near its binding sites, neither is required to be free for optimum activity.

L53 ANSWER 4 OF 9 CAPLUS COPYRIGHT 1998 ACS
AN 1994:595333 CAPLUS
DN 121:195333
TI Mechanisms of dichotomous action of IL-2-Pseudomonas exotoxin 40 (IL-2-PE40) on cell-mediated and humoral immune response
AU Volk, Hans-Dieter; Muller, Sabine; Yarkoni, Shai; Diamantstein, Tibor; Lorberbaum-Galski, Haya
CS Institute of Medical Immunology, Humboldt University of Berlin, Germany
SO J. Immunol. (1994), 153(6), 2497-505
CODEN: JOIMA3; ISSN: 0022-1767
DT Journal
LA English
AB IL-2-PE40 is a chimeric protein composed of human IL-2 genetically fused to the **N terminus** of a **modified** form of Pseudomonas exotoxin lacking its cell recognition domain. The immunosuppressive efficacy of IL-2-PE40 was demonstrated in several exptl. murine transplant and autoimmune models. However, some observations suggested that IL-2-PE40 could not inhibit the humoral response. In this report, the authors describe the dichotomous effects of IL-2-PE40 on humoral and cell-mediated immune response in a simple, well characterized in vivo model. Although IL-2-PE40 inhibited the cell-mediated delayed type hypersensitivity reaction to SRBC, it increased the humoral immune response to the same antigen. To understand the mechanism of dichotomous action of IL-2-PE40 on the immune response, IL-2R-bearing T cells were treated with IL-2-PE40 in vitro and the cytokine expression was studied at mRNA and protein level. Similar to IL-2, IL-2-PE40 promoted the expression of T helper 1-like (IFN-.gamma.) as well as T helper 2-like (**IL-4**, IL-10) cytokines. These in vitro studies show that IL-2-PE40 can induce signal transduction in activated T cells through the IL-2R before exerting its cytotoxic effect. In contrast to DTH reaction, humoral immune response requires T cell help only for a limited period. Therefore, the short-term stimulation of T helper cells by IL-2-PE40 may be sufficient in vivo to mediate a B cell response in the local environment, whereas the DTH reaction and other cell-mediated immune responses are inhibited by the toxin moiety of the chimeric protein.

L53 ANSWER 5 OF 9 CAPLUS COPYRIGHT 1998 ACS
AN 1994:105024 CAPLUS
DN 120:105024
TI Mutant cytokines having increased receptor affinity
IN Lakkis, Fadi; Murphy, John R.
PA University Hospital, USA
SO PCT Int. Appl., 28 pp.
CODEN: PIXXD2
PI WO 9321308 A1 931028
DS W: AT, AU, BB, BG, BR, CA, CH, CZ, DE, DK, ES, FI, GB, HU, JP, KP, KR, LK, LU, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SK,
Searched by Barb O'Bryen, STIC 308-4291

UA, VN

RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FR, GA, GB, GR,
IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG

AI WO 93-US3613 930416

PRAI US 92-870500 920417

DT Patent

LA English

AB A variant of a naturally-occurring cytokine has a neutral amino acid substituted for a neg.-charged amino acid within 2 amino acids immediately upstream or downstream from a Phe-Leu or Tyr-Leu sequence in a helical domain. The variant cytokine has an increased affinity for the receptor. A hybrid mol. comprises a receptor-binding portion of the variant cytokine joined together covalently with a mol. having enzymic activity (e.g., a cytotoxin). The hybrid mol. decreases cell viability. DAB389-mIL-4, a fusion protein contg. diphtheria toxin having a deletion of 97 amino acids (Thr387-His485; the generalized cell binding domain) replaced with murine IL-4, was altered by site-directed and in-frame deletion mutagenesis to alter the mIL-4 portion of DAB389-mIL-4. Deletion of the C-terminal 15 amino acids of mIL-4; substitution of Phe496 with Pro, Ala, or Tyr; or substitution of Leu497 with Ala or Glu decreased binding to the mIL-4 receptor and cytotoxicity. In contrast, the substitution of the neg.-charged residue Asp495 with Asn resulted in a 4-fold increase in cytotoxic potency and binding affinity to mIL-4 receptor bearing cells in vitro.

L53 ANSWER 6 OF 9 CAPLUS COPYRIGHT 1998 ACS

AN 1992:468221 CAPLUS

DN 117:68221

TI A receptor binding domain of mouse interleukin-4 defined by a solid-phase binding assay in vitro mutagenesis

AU Morrison, Briggs W.; Leder, Philip

CS Dep. Genet., Harvard Med. Sch., Boston, MA, 02115, USA

SO J. Biol. Chem. (1992), 267(17), 11957-63

CODEN: JBCHA3; ISSN: 0021-9258

DT Journal

LA English

AB Interleukin 4 (IL-4) is a potent, pleiotropic lymphokine that affects a variety of cells, esp. those of hematopoietic origin. Although murine and human IL-4 are homologous proteins, they display a species specificity in which murine IL-4 acts only upon mouse cells, and human IL-4 only upon human cells. A mutagenesis strategy was used to define both the structural determinants of this specificity and a receptor binding domain of murine IL-4. To do this, convenient solid-phase binding assays for mouse and for human IL-4 were developed, each utilizing receptor-Ig fusion proteins and alk. phosphatase-tagged ligands. These were employed to assess the receptor binding activities of wild type and mutant forms of IL-4. In a sep. biol. assay, the ability was measured of each version of IL-4 to induce proliferation of a cultured mouse T-cell line. By replacing regions of mouse IL-4 with homologous segments of human IL-4, it was found that the N-terminal 16 residues and the C-terminal 20 residues of murine IL-4 are required for species-specific receptor binding as well as for T-cell proliferation. A major portion of the amino acid sequence between these regions can be substituted between mouse and human without loss of receptor binding or biol. activity. Further, Searched by Barb O'Bryen, STIC 308-4291

alanine-scanning mutagenesis revealed specific residues in the N- and C-terminal regions (Glu-12, Ile-14, Leu-104, Asp-106, Phe-107, and Leu-111) that bear side chains crit. for function. An anal. of the C-terminal region of murine IL-4 and its comparison with C-terminal regions of other related cytokines suggest an evolutionary conservation of structural and functional features.

L53 ANSWER 7 OF 9 CAPLUS COPYRIGHT 1998 ACS
AN 1993:37265 CAPLUS
DN 118:37265
TI Native and recombinant soluble CD23 fragments with IgE suppressive activity
AU Sarfati, M.; Bettler, B.; Letellier, M.; Fournier, S.;
Rubio-Trujillo, M.; Hofstetter, H.; Delespesse, G.
CS Res. Cent., Notre-Dame Hosp., Montreal, PQ, H2L 4M1, Can.
SO Immunology (1992), 76(4), 662-7
CODEN: IMMUAM; ISSN: 0019-2805
DT Journal
LA English
AB CD23-bearing cells release 37,000, 33,000, and 25,000 MW sol. CD23 (sCD23) fragments that were reported to display multiple biol. activities, including the potentiation of IgE synthesis. It was previously reported that tunicamycin treatment of RPMI-8866 cells switched the biol. activity of the sCD23 released by these cells from IgE potentiation to IgE suppression. Here it is shown that tunicamycin-treated cells release small CD23 fragments with a MW of 16,000. These fragments are formed by truncation of the N-terminal 160 amino acids and truncation of the C-terminal end of CD23. Two observations indicate that the cleavage of surface CD23 into 16,000 MW fragments is not caused by tunicamycin-mediated inhibition of the N-glycosylation of CD23 but rather by the deletion of the C-terminal end of the mol.: (1) CHO transfectants expressing a CD23 **mutant** lacking the N-glycosylation site release 37,000-33,000 MW sCD23 unless they are treated with tunicamycin; (2) transfectants expressing a CD23 deletion **mutant** lacking the last 33 C-terminal amino acids release 16,000 MW sCD23. Highly purified native and recombinant 16,000 MW sCD23 fragments bind to IgE and down-regulate the ongoing and the **interleukin-4 (IL-4)** -stimulated synthesis of IgE.

L53 ANSWER 8 OF 9 WPIDS COPYRIGHT 1998 DERWENT INFORMATION LTD
AN 91-088700 [13] WPIDS
DNC C91-037677
TI Nucleic acid encoding mammalian **interleukin-4 receptor** - used as **antagonists of interleukin-4** in treating conditions associated with excess IgE prodn. including allergic conditions.
DC B04 D16
IN GALIZZI, J; HARADA, N; MIYAJIMA, A; GALIZZI, J P; MAYAJIMA, A
PA (SCHE) SCHERING CORP
CYC 32
PI EP 419091 A 910327 (9113)*
R: GR
WO 9103555 A 910321 (9114)
RW: AT BE CH DE DK ES FR GB IT LU NL OA SE
W: AU BB BG BR FI HU JP KP KR LK MC MG MW NO RO SD SU
AU 9064122 A 910408 (9127)
FI 9200987 A 920306 (9223)
EP 490975 A1 920624 (9226) EN 28 pp
R: AT BE CH DE DK ES FR GB IT LI LU NL SE
Searched by Barb O'Bryen, STIC 308-4291

NO 9200900 A 920306 (9226)
 JP 04504061 W 920723 (9236) 10 pp
 HU 62935 T 930628 (9326)
 AU 641941 B 931007 (9346)

ADT EP 419091 A EP 90-309700 900905; FI 9200987 A WO 90-US4949 900905,
 FI 92-987 920306; EP 490975 A1 EP 90-913924 900905, WO 90-US4949
 900905; NO 9200900 A WO 90-US4949 900905, NO 92-900 920306; JP
 04504061 W JP 90-513019 900905, WO 90-US4949 900905; HU 62935 T WO
 90-US4949 900905, HU 92-773 900905; AU 641941 B AU 90-64122 900905

FDT EP 490975 A1 Based on WO 9103555; JP 04504061 W Based on WO 9103555;
 HU 62935 T Based on WO 9103555, Based on WO 9103555; AU 641941 B
 Previous Publ. AU 9064122, Based on WO 9103555

PRAI US 89-404179 890907; US 90-496449 900320

AB EP 419091 A UPAB: 940120

The nucleic acid pref. encodes a soluble form of human **interleukin-4** receptor comprising an extracellular domain of the receptor. The extracellular domain comprises the 200-220 N-terminal amino acids of the human **interleukin-4** receptor. Also claimed is the soluble human **interleukin-4** receptor comprising the extracellular domain and a plasmid pME185-hIL-4R deposited at the ATTC under accession no. 682263. Soluble forms of **interleukin-4** may be produced by introducing a stop codon prior to the coding region for the transmembrane and intracellular portions of the **interleukin-4** receptor cDNA. The **interleukin-4** receptor may be administered by continuous infusion so that an amt. of 50-800 micron is delivered per day.

USE/ADVANTAGE - Nucleic acid is used as an **antagonist** for related receptors by cross hybridisation and the soluble forms of human **interleukin-4** receptors are used as **antagonists of interleukin-4** in the treatment of conditions associated with excessive IgE prodn. including allergic conditions and immune disorders. @19pp
 Dwg.No.0/5)@

L53 ANSWER 9 OF 9 WPIDS COPYRIGHT 1998 DERWENT INFORMATION LTD
 AN 90-067162 [09] WPIDS
 DNC C90-029378
 TI Compsns. contg. recombinant non-glycosylated human interleukin-3 - has increased biological activity and binding affinity, for treating cytopenias.
 DC B04 D16
 IN ANDERSON, D M; COSMAN, D J; PRICE, V L
 PA (IMMV) IMMUNEX CORP
 CYC 15
 PI WO 9001039 A 900208 (9009)* EN 23 pp
 RW: AT BE CH DE FR GB IT LU NL SE
 W: AU DK JP NO
 AU 8938640 A 900219 (9030)
 EP 425536 A 910508 (9119)
 R: AT BE CH DE FR GB IT LI LU NL SE
 JP 04500508 W 920130 (9211) 23 pp
 EP 425536 A4 920102 (9520)
 ADT WO 9001039 A WO 89-US2599 890614; EP 425536 A EP 89-907981 890614;
 JP 04500508 W JP 89-507356 890614; EP 425536 A4 EP 89-907981
 PRAI US 88-221699 880720
 AB WO 9001039 A UPAB: 930928
 A pharmaceutical compsn. contains an effective amt. of a recombinant human interleukin-3 protein analogue, rhu IL-3, (Asp15, Asp70), which is **derived** by yeast expression and which has the following properties: (a) a biological specific activity of at least Searched by Barb O'Bryen, STIC 308-4291

3.0 x 10 power 7 U/mg in a human bone marrow proliferation assay;
(b) a binding affinity for human monocyte IL-3 receptors, expressed
as an inhibition constant, of at least 2.0 x 10 power 10 M-1; and
(c) an endotoxin activity of less than 1ng/mg protein.

The compsn. may also comprise the following **N-terminal** octapeptide: D-Y-K-D-D-D-D-K. It may also contain a physiologically acceptable diluent and at least one biological response **modifier** from IL-1X, IL-1B, IL-2, **IL-4**, IL-5, IL-6, IL-7, GM-CSF, G-CSF, M-CSF, EPO, or TNF.

USE/ADVANTAGE - The rhuIL-3 analogues have increased biological activity and binding affinity relative to wild type proteins. The new proteins may be used in the treatment of various cytopenias. Either alone, or in combination with other lympholines they may be used to potentiate immune responsiveness to infectious pathogens, or to help in reconstituting blood cell populations following viral infection or radiation- or chemotherapy-induced haematopoietic cell suppression.

0/4

=> fil wpids; d que 149 ; fil capl; d que 134

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DERWENT WEEK FOR POLYMER INDEXING: 199801
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L41 73 SEA FILE=WPIDS ABB=ON L40 NOT PY>1995
L43 78 SEA FILE=WPIDS ABB=ON L40(L) (MUTANT? OR MUTAT? OR MUTEIN?
? OR DERIV? OR REPLAC? OR SUBSTITUT? OR MODIF? OR AGONIST?
? OR ANTAGONIST?)
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L49 0 SEA FILE=WPIDS ABB=ON L43 AND L47 AND L41

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L7 8689 SEA FILE=CAPLUS ABB=ON L6 OR IL4
L8 211758 SEA FILE=CAPLUS ABB=ON MUTANT? OR MUTAT? OR MUTEIN?
L9 508178 SEA FILE=CAPLUS ABB=ON DERIVATIVE#
L10 1066629 SEA FILE=CAPLUS ABB=ON REPLAC? OR SUBSTITUT? OR MODIF?
L11 168570 SEA FILE=CAPLUS ABB=ON AGONIST? OR ANTAGONIST?
L12 1097 SEA FILE=CAPLUS ABB=ON L7(L) ((L8 OR L9 OR L10 OR L11))
L17 288781 SEA FILE=CAPLUS ABB=ON TERMIN?
L19 60766 SEA FILE=CAPLUS ABB=ON (C OR CARBOX?) (2A)L17
L32 15 SEA FILE=CAPLUS ABB=ON L19(S) ((L8 OR L9 OR L10 OR L11))
AND L12
L34 8 SEA FILE=CAPLUS ABB=ON L32 NOT PY>1995

=> s 134 not (136 or 120)

L55 3 L34 NOT (L36 OR L20) *previously run*

=> d bib ab 155 1-3

L55 ANSWER 1 OF 3 CAPLUS COPYRIGHT 1998 ACS

AN 1995:507361 CAPLUS

DN 122:263278

TI Species-specific **agonist/antagonist** activities
of human **interleukin-4** variants suggest distinct
ligand binding properties of human and murine common receptor
.gamma. chain

AU Boensch, Dominikus; Kammer, Winfried; Lischke, Antje; Friedrich,
~~Karlheinz~~

CS Theodor-Boveri-Inst. Biowissenschaften, Univ. Wuerzburg, Wuerzburg,
D-97074, Germany

SO J. Biol. Chem. (1995), 270(15), 8452-7
CODEN: JBCHA3; ISSN: 0021-9258

DT Journal

LA English

AB **Interleukin-4 (IL-4)** is a pleiotropic cytokine eliciting various responses in target cells upon binding to its receptor. Activation of the **IL-4** receptor probably involves interaction of the ligand with both the **IL-4** receptor .alpha. subunit (IL-R.alpha.) and the common .gamma. chain (c.gamma.). Although human and murine **IL-4** receptor .alpha. chains are specific for **IL-4** from the same species, murine c.gamma. can form a signal-competent complex with human IL-4R.alpha. (hIL-4R.alpha.) and human **IL-4** (hIL-4). We have generated a hIL-4 responsive murine myeloid cell line (FDC-4G) expressing a chimera comprising the extracellular domain of human IL-4R.alpha. and the intracellular domain of human granulocyte colony-stimulating factor receptor (hG-CSFR). This hybrid receptor was shown to form a complex with hIL-4 and the murine c.gamma.-chain. Biol. activities of human **IL-4** variants on murine FDC-4G cells and on the human erythroleukemic cell line TF-1 displayed a strikingly different pattern. Single amino acid **replacements** at two different positions in the **C-terminal** helix of hIL-4, the region of the previously defined "signaling site," lead to an **inverse agonist/antagonist** behavior of the resulting cytokines in the two cellular systems. From these findings we conclude that upon formation of the activated **IL-4** receptor complex murine and human c.gamma. interact with hIL-4 in a geometrically different fashion.

L55 ANSWER 2 OF 3 CAPLUS COPYRIGHT 1998 ACS

AN 1995:494173 CAPLUS

DN 122:258386

TI Importance of the glutamate residue of KDEL in increasing the cytotoxicity of Pseudomonas exotoxin derivatives and for increased binding to the KDEL receptor

AU Kreitman, Robert J.; Pastan, Ira

CS Lab. Mol. Biol., Natl. Cancer Inst., Bethesda, MD, 20892, USA

SO Biochem. J. (1995), 307(1), 29-37

CODEN: BIJOAK; ISSN: 0264-6021

DT Journal

LA English

AB It was previously shown that amino acids 609-613 (REDLK) at the Searched by Barb O'Bryen, STIC 308-4291

C-terminus of Pseudomonas exotoxin (PE) are necessary for cytotoxicity, presumably by directing the toxin to the endoplasmic reticulum (ER) (V. K. Chaudhary et al.; 1990). Using the anti-[interleukin 2 receptor (IL2R)] immunotoxin anti-Tac(Fv)-PE38 (AT-PE38REDLK), it was found that removing the terminal lysine did not alter the activity, but replacing REDL with KDEL, the most common ER retention sequence, increased activity. To determine which amino acid in KDEL was responding for the increase in activity, we tested eight C-terminal mutants of AT-PE38REDLK. Using IL2R-bearing MT-1 cells, we found that the glutamate residue of KDEL was required for high activity, as the cytotoxicity of AT-PE38 ending in KDEL, RDEL, KEEL or REEL was much greater than that of AT-PE38 ending in REDL, KEDL, RDDL or KDDL. Using freshly isolated lymphocytic leukemia cells, AT-PE38 ending in KDEL, REEL or RDEL was more than 100-fold more cytotoxic than AT-PE38 ending in KEDL, REDL, RDDL or the native sequence REDLK. The RDEL sequence also improved the cytotoxic activity of an interleukin 4-PE38 toxin fusion protein. Improved cytotoxic activity correlated with improved binding of the C-termini to the KDEL receptor on rat Golgi membranes. These data indicate that the glutamate residue of KDEL improves the cytotoxicity of PE by increasing binding to a sorting receptor which transports the toxin from the transreticular Golgi app. to the ER, where it is translocated to the cytosol and inhibits protein synthesis.

L55 ANSWER 3 OF 3 CAPLUS COPYRIGHT 1998 ACS
AN 1994:577324 CAPLUS
DN 121:177324
TI Site-Specific Conjugation to **Interleukin 4**
Containing **Mutated** Cysteine Residues Produces
Interleukin 4-Toxin Conjugates with Improved
Binding and Activity 77
AU Kreitman, Robert J.; Puri, Raj K.; Leland, Pamela; Lee, Byungkook;
Pastan, Ira
CS Division of Cancer Biology, National Cancer Institute, Bethesda, MD,
20892, USA
SO Biochemistry (1994), 33(38), 11637-44
CODEN: BICHAW; ISSN: 0006-2960
DT Journal
LA English
OS CJACS-IMAGE; CJACS
AB Fusion of a ligand to another protein frequently impairs the binding of the ligand. Recombinant toxins composed of **mutants** of Pseudomonas exotoxin (PE) fused to the **C-terminus** of human **interleukin 4** (**IL4**) are cytotoxic to **IL4** receptor- (**IL4R**) bearing tumor cells but bind to the **IL4R** with only 1% the affinity of **IL4**. The authors have developed a method to connect a toxin to a ligand which allows the junction to be moved to a location on the ligand which would minimize the binding impairment. The authors designed **mutants** of **IL4** in which residue 28, 38, 68, 70, 97, or 105 was **substituted** with cysteine. All purified **mutants** bound to the **IL4R** with 60-100% the affinity of **IL4**, indicating that the **IL4** structure was essentially unchanged. The **IL4** **mutants** were then each conjugated through a disulfide bond to PE35, a truncated form of PE which contains a single cysteine. **IL4** conjugated to PE35 at residue 28, 38, or 105 of **IL4** bound with 10-fold improved affinity and was 10-fold more cytotoxic than the recombinant **IL4**-toxin in which PE is fused to position 129 at the C-terminus of **IL4**. **IL4** contg. PE35
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conjugated at position 68, 70, or 97 had lower binding affinity and cytotoxic activity. These results indicate that the location of the ligand-protein junction can be selectively moved to enhance conjugate effectiveness, and implications could be made regarding which regions of **IL4** are important for binding.

=> fil capl; d que 126

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L8 211758 SEA FILE=CAPLUS ABB=ON MUTANT? OR MUTAT? OR MUTEIN?
L9 508178 SEA FILE=CAPLUS ABB=ON DERIVATIVE#
L10 1066629 SEA FILE=CAPLUS ABB=ON REPLAC? OR SUBSTITUT? OR MODIF?
L11 168570 SEA FILE=CAPLUS ABB=ON AGONIST? OR ANTAGONIST?
L12 1097 SEA FILE=CAPLUS ABB=ON L7(L) ((L8 OR L9 OR L10 OR L11))
L23 23299 SEA FILE=CAPLUS ABB=ON GLYCOSYLAT?
L25 12 SEA FILE=CAPLUS ABB=ON L12 (L) L23
L26 8 SEA FILE=CAPLUS ABB=ON L25 NOT PY>1995

=> s 126 not (120 or 136 or 134)

L57 7 L26 NOT (L20 OR L36 OR L34)

=> fil wpids; d que 151 previously printed

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L43 78 SEA FILE=WPIDS ABB=ON L40(L) (MUTANT? OR MUTAT? OR MUTEIN
? OR DERIV? OR REPLAC? OR SUBSTITUT? OR MODIF? OR AGONIST
? OR ANTAGONIST?)
L50 1053 SEA FILE=WPIDS ABB=ON GLYCOSYLAT?
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L51 3 SEA FILE=WPIDS ABB=ON L43 AND L50 AND L41

=> s 151 not 148

L59 2 L51 NOT L48 *previously printed*

=> dup rem 157,159

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L60 8 DUP REM L57 L59 (1 DUPLICATE REMOVED)

=> d bib ab 160 1-8; fil hom

L60 ANSWER 1 OF 8 CAPLUS COPYRIGHT 1998 ACS

AN 1994:131568 CAPLUS

DN 120:131568

TI Production of large amounts of recombinant interleukins by cDNA transfected mouse myeloma cells cultured in dialysis tubing

AU Sjoegren-Jansson, Eva; Gustafsson, Carolina; Jeansson, Stig; Karlsson, Ulla; Lycke, Nils

CS Clinical Virology and, Goteborg, S-413 46, Swed.

SO J. Immunol. Methods (1994), 168(1), 131-6

CODEN: JIMMBG; ISSN: 0022-1759

DT Journal

LA English

AB Studies of interleukin function often require large quantities of these highly expensive substances. The available interleukins are generally recombinant proteins produced in bacteria or yeast and, less commonly, interleukins produced by mammalian cells, which provide appropriate glycosylation and other post-translational modifications. Due to differences in biosynthesis, difficulties in prodn. and purifn. the quality of the interleukin preps. may vary. The authors have taken advantage of the recently developed constitutively interleukin-secreting mouse myeloma cell lines and the dialysis tubing culture technique, which permit cells to be grown at high densities, in order to establish a method for the prodn. of large amts. of recombinant murine IL-2 and IL-4. These interleukins can be produced at low cost and in concns. 20-30-fold higher than in conventional culture flasks. A single dialysis tubing culture will produce more than 10⁶ U of interleukin which may be compared with the available com. preps. contg. between 10- and a 100-fold less per vial. The IL-2 and IL-4 produced in this manner are biol. active mols. as demonstrated by the strong proliferative response of clonal T cells and the isotype-switching effect in LPS-stimulated splenic B cell cultures. The dialysis tubing culture technique is a simple and highly cost-effective means of generating large quantities of biol. active interleukins and is esp. suitable for research labs. interested in functional studies of these proteins.

L60 ANSWER 2 OF 8 CAPLUS COPYRIGHT 1998 ACS

AN 1995:286861 CAPLUS

DN 122:78834

TI Cytokine and growth factor regulation of macrophage scavenger receptor expression and function
AU de Villiers, Willem J. S.; Fraser, Iain P.; Gordon, Siomon
CS Sir William Dunn School of Pathology, University of Oxford, South Parks Road, Oxford, OX1 3RE, UK
SO Immunol. Lett. (1994), 43(1,2), 73-9
CODEN: IMLED6; ISSN: 0165-2478
DT Journal
LA English
AB Regulation of macrophage scavenger receptor (MSR) activity may be an important determinant of the extent of atherogenesis and the efficacy of host defense. The effect of M-CSF on this pathway was studied using a recently developed monoclonal antibody to murine MSR. M-CSF markedly and selectively increased MSR synthesis in murine macrophages (M.PHI.); post-translationally the receptor appeared more stable and shifted to a predominantly surface distribution. Functionally M-CSF enhanced **modified** lipoprotein uptake and increased divalent cation-independent adhesion in vitro. These results suggest a plausible mechanism whereby M-CSF prodn. in the atheromatous plaque micro-environment could promote the recruitment and retention of mononuclear phagocytes and subsequent foam cell formation. In addn., the Th1 cytokine (.gamma.-interferon) and Th2 cytokine (**interleukin -4**) had differential effects on MSR **glycosylation** in vitro suggesting a further possible regulatory role by these lymphokines on macrophage MSR function.

L60 ANSWER 3 OF 8 CAPLUS COPYRIGHT 1998 ACS
AN 1994:296353 CAPLUS
DN 120:296353
TI Peripheral blood neutrophil production of interleukin-1 receptor antagonist and interleukin-1.beta.
AU Malyak, Mark; Smith, Michael F.; Abel, Ashley A.; Arend, William P.
CS Health Sci. Cent., Univ. Colorado, Denver, CO, 80262, USA
SO J. Clin. Immunol. (1994), 14(1), 20-30
CODEN: JCIMDO; ISSN: 0271-9142
DT Journal
LA English
AB Interleukin-1 receptor **antagonist** (IL-1ra) and interleukin-1.beta. (IL-1.beta.) prodn. by human peripheral blood neutrophils (PMN) was studied. Unstimulated PMN contained IL-1ra protein in the absence of IL-1ra mRNA; IL-1.beta. mRNA and protein were undetectable. Lipopolysaccharide (LPS), granulocyte/macrophage colony-stimulating factor (GM-CSF), and tumor necrosis factor-alpha (TNF-.alpha.), individually, transiently increased IL-1ra steady-state mRNA levels in PMN, with assocd. increases in IL-1ra protein synthesis. LPS, GM-CSF, and TNF-.alpha. generated similar increases in IL-1.beta. mRNA, yet only LPS resulted in detectable synthesis of IL-1.beta. protein. **IL-4** enhanced LPS-induced IL-1ra prodn. by PMN and inhibited LPS-induced IL-1.beta. prodn. IL-1ra protein present within stimulated PMN supernatants existed in the 22-25 kDa **glycosylated** form. Polymerase chain reaction amplification detd. that only secretory IL-1ra mRNA was present within stimulated PMN; intracellular IL-1ra mRNA was undetectable. Thus, freshly isolated PMN possess a small amt. of IL-1ra protein and these cells can respond to stimuli with a low level of sIL-1ra transcription and translation. PMN may be a major source of IL-1ra in inflammatory exudates where these cells predominate.

L60 ANSWER 4 OF 8 CAPLUS COPYRIGHT 1998 ACS
AN 1993:407002 CAPLUS
Searched by Barb O'Bryen, STIC 308-4291

DN 119:7002
TI Regulation of interleukin-1ra, interleukin-1.alpha., and interleukin-1.beta. production by human alveolar macrophages with phorbol myristate acetate, lipopolysaccharide, and interleukin-4
AU Galve-De Rochemonteix, Beatrice; Nicod, Laurent P.; Chicheportiche, Rachel; Lacraz, Sylvie; Baumberger, Christophe; Dayer, Jean Michel
CS Div. Immunol. Allergy, Univ. Hosp., Geneva, 1211, Switz.
SO Am. J. Respir. Cell Mol. Biol. (1993), 8(2), 160-8
CODEN: AJRBEL; ISSN: 1044-1549
DT Journal
LA English
AB Human alveolar macrophages (AM) are antigen-presenting cells that have an important immune effector function in the lung. It was previously shown that AM produce a specific interleukin-1 (IL-1) inhibitor of 20 to 25 kD that blocks biol. activities of IL-1.alpha. and IL-1.beta. such as prostaglandin E2 prodn. by fibroblasts. This inhibitor acts as a receptor **antagonist** (IL-1ra) by binding to the IL-1 receptor. This paper presents evidence that the natural AM-derived IL-1ra is immunol. identical to IL-1ra cloned from human peripheral blood monocytes and shows a band at 20 kD compatible with the natural **glycosylated** IL-1ra. No constitutive expression of IL-1 mRNA was detected when analyzed by Northern blot immediately after bronchoalveolar lavage from six control patients. Comparison of in vitro kinetics of IL-1ra, IL-1.alpha., and IL-1.beta. analyzed during culture in the presence or absence of phorbol myristate acetate revealed that their mRNA expression was asynchronous. IL-1.alpha. and IL-1.beta. mRNA were expressed after as little as 15 min, whereas IL-1ra mRNA was detectable only after 3 h in culture. The prodn. of IL-1ra was measured by ELISA and compared with that of IL-1.alpha. and IL-1.beta.. In freshly isolated AM (106/mL), cell-assocd. IL-1ra was present in an av. amt. of 2.0 ng/mL, i.e., 25 and 100 times more than IL-1.alpha. and IL-1.beta., resp. After 24 h of culture, the amt. of extracellular IL-1ra was equal to that of intracellular IL-1ra and was 30 and 100 times higher than IL-1.alpha. or IL-1.beta., resp. In the presence of **IL-4**, a clear dissociation between IL-1.alpha. or IL-1.beta. and IL-1ra prodn. was obsd., the prodn. of IL-1ra being increased whereas that of IL-1.alpha. or IL-1.beta. was decreased or unchanged. The results show that the kinetics of IL-1ra, IL-1.alpha., and IL-1.beta. prodn. differ and that cytokines such as **IL-4** favor the prodn. of IL-1ra at the transcriptional level to preserve the lung from inflammation.

L60 ANSWER 5 OF 8 CAPLUS COPYRIGHT 1998 ACS
AN 1993:166846 CAPLUS
DN 118:166846
TI Expression of murine soluble CD4 protein in baculovirus infected insect cells
AU Kupsch, Jorg; Saizawa, Kai M.; Eichmann, Klaus
CS Max-Planck-Inst. Immunbiol., Freiburg, Germany
SO Immunobiology (Stuttgart) (1992), 186(3-4), 254-67
CODEN: IMMND4; ISSN: 0171-2985
DT Journal
LA English
AB The expression of murine sol. CD4 (L3T4) protein (sCD4) by baculovirus-infected insect cells was characterized. The yield of sCD4 reached 2 mg/L culture supernatant late in infection. Nevertheless, a large amt. of sCD4 remained cell-assocd., presumably in the endoplasmic reticulum or an early golgi compartment, as indicated by the endo-.beta.-N-acetyl-D-glucosaminidase H (endo-H) sensitivity of its carbohydrate chains. The secreted form of sCD4
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is modified with both endo-.beta.-N-acetyl-D-glucosaminidase D (endo-D) and endo-H-sensitive oligosaccharides. It was possible that the incomplete secretion indicated faulty glycosylation or improper folding of the sCD4 protein. However, inhibitor studies showed that complete carbohydrate processing is not required for secretion of sCD4 by insect cells. Moreover, maintained reactivity with a panel of monoclonal Ab as well as phase partitioning expts. suggested that secretion is apparently not caused by misfolding of the sCD4 protein. Similar results were obtained with biol. active murine interleukin-4 produced by insect cells. Thus, an inefficient secretory pathway may be a general problem of baculovirus-infected insect cells and is not a consequence of incorrect mol. conformation.

L60 ANSWER 6 OF 8 WPIDS COPYRIGHT 1998 DERWENT INFORMATION LTD
AN 89-131926 [18] WPIDS
CR 87-150613 [21]; 91-156128 [21]
DNN N89-100476 DNC C89-058363
TI Monoclonal antibody specific for human interleukin-4 - used to detect, purify and measure the concn. or as agonists or antagonists of interleukin-4.
DC B04 D16 S03
IN ABRAMS, J S; CHRETIEN, I; LEE, F D; PEARCE, M K; LEE, F
PA (SCHE) SCHERING BIOTECH CORP; (SCHE) SCHERING-BIOTECH CO
CYC 28
PI EP 314402 A 890503 (8918)* EN 15 pp
R: ES GR
WO 8903846 A 890505 (8920) EN
RW: AT BE CH DE FR GB IT LU NL OA SE
W: AU DK FI HU JP KR NO US
PT 88847 A 890731 (8935)
AU 8927827 A 890523 (8939)
CN 1032816 A 890510 (9017)
EP 375743 A 900704 (9027)
R: AT BE CH DE FR GB IT LI LU NL SE
ZA 8807987 A 900627 (9030)
DK 9001018 A 900425 (9037)
JP 03503118 W 910718 (9135)
US 5041381 A 910820 (9136)
IL 88145 A 930922 (9349)
EP 375743 B1 931229 (9401) EN 23 pp
R: AT BE CH DE ES FR GB GR IT LI LU NL SE
DE 3886760 G 940210 (9407)
KR 9308112 B1 930826 (9432)
JP 06098020 B2 941207 (9502) 14 pp
ES 2061736 T3 941216 (9505)
DK 169627 B 941227 (9506)
IE 62491 B 950208 (9518)
CA 1335653 C 950523 (9528)
ADT EP 314402 A EP 88-309926 881021; WO 8903846 A WO 88-US3631 881021;
EP 375743 A EP 88-910302 881021; ZA 8807987 A ZA 88-7987 881025; JP
03503118 W JP 88-509356 881021; US 5041381 A US 87-113623 871026; IL
88145 A IL 88-88145 881025; EP 375743 B1 EP 88-910302 881021, WO
88-US3631 881021; DE 3886760 G DE 88-3886760 881021, EP 88-910302
881021, WO 88-US3631 881021; KR 9308112 B1 WO 88-US3631 881021, KR
89-701139 890623; JP 06098020 B2 JP 88-509356 881021, WO 88-US3631
881021; ES 2061736 T3 EP 88-910302 881021; DK 169627 B WO 88-US3631
881021, DK 90-1018 900425; IE 62491 B IE 88-3213 881025; CA 1335653
C CA 88-581117 881025
FDT EP 375743 B1 Based on WO 8903846; DE 3886760 G Based on EP 375743,
Searched by Barb O'Bryen, STIC 308-4291

Based on WO 8903846; JP 06098020 B2 Based on JP 03503118, Based on WO 8903846; ES 2061736 T3 Based on EP 375743; DK 169627 B Previous Publ. DK 9001018

PRAI US 87-113623 871026; US 85-799668 851119; US 86-843958 860325;
US 86-881553 860703

AB EP 314402 A UPAB: 961007

A monoclonal antibody (MAb) specific for human **interleukin-4** (hIL-4) is claimed. Also claimed is a hybridoma capable of secreting MAb specific for hIL-4. The hybridomas and MBbs are produced against either **glycosylated** or unglycosylated versions of recombinantly-produced mature hIL-4. Antibodies and antibody fragments characteristic of the hybridomas can also be produced recombinantly by extracting mRNA, constructing a cDNA library and selecting clones which encode segments of the antibody molecule.

USE - The MBbs can be used to detect, purify and measure the concn. of hIL-4. They may also be useful as **agonists** or **antagonists** of hIL-4 and used against **IL-4**-related diseases, eg as therapeutic agents for treating atopic diseases.

Dwg.0/3

Dwg.0/3

L60 ANSWER 7 OF 8 CAPLUS COPYRIGHT 1998 ACS
AN 1990:113525 CAPLUS

DN 112:113525

TI Expression of human and murine interleukin-5 in eukaryotic systems

AU Tavernier, Jan; Devos, Rene; Van der Heyden, Jose; Hauquier, Guido; Bauden, Rita; Fache, Ina; Kawashima, Eric; Vandekerckhove, Joel; Contreras, Roland; Fiers, Walter

CS Roche Res. Gent, Ghent, B-9000, Belg.

SO DNA (1989), 8(7), 491-501

CODEN: DNAADR; ISSN: 0198-0238

DT Journal

LA English

AB A cDNA coding for murine interleukin-5 (IL-5) was isolated from the EL4.ExC5 cell line. With the exception of a single amino acid **substitution** at position 79 (Arg.fwdarw.His), it is identical to a published sequence. The coding sequence for human IL-5 was synthesized chem., allowing the introduction of strategically located restriction enzyme cleavage sites. Both cDNAs were expressed in various eukaryotic systems. Deletion of the 3' untranslated region of the murine IL-5 gene led to a 5-10-fold increase in expression in *Xenopus laevis* oocytes and in NIH-3T3 cells. The highest prodn., however, was obtained in Sf9 cells using a baculovirus vector. Human IL-5 was obtained from transformed *Saccharomyces cerevisiae* as a secreted, mature form using an in-frame fusion to the leader sequence of .alpha.-mating type factor, and was purified to homogeneity. In all cases mentioned, IL-5 was found to be **glycosylated**, and its biol. activity was dependent on a 40- to 50-kD homodimer configuration, linked together by disulfide bridges. Deglycosylation did not affect the biol. activity. Recombinant human IL-5 is biol. active on some human B-CLL cells (proliferation in the presence of IL-2) and on murine BC11 cells (proliferation) at a low specific activity (about 1-2 .times. 10³ U/mg) and on human eosinophils (eosinophil peroxidase assay) at a high specific activity (at least 5 .times. 10⁶ U/mg). Recombinant murine IL-5 from S49 cells has a specific activity of 1-2 .times. 10⁷ U/mg in the BC11 proliferation assay. An additive effect is seen in the presence of murine granulocyte-macrophage colony-stimulating factor (GM-CSF) and a synergistic effect in the presence of murine **IL-4**.

Searched by Barb O'Bryen, STIC 308-4291

L60 ANSWER 8 OF 8 CAPLUS COPYRIGHT 1998 ACS DUPLICATE 1
AN 1989:226601 CAPLUS
DN 110:226601
TI Construction and expression in interleukin-4-mutein-encoding plasmids, and use of interleukin 4 to induce proliferation of cytotoxic T lymphocytes
IN Anderson, Dirk M.; Cosman, David J.; Deeley, Michael C.; Grabstein, Kenneth H.; Price, Virginia L.
PA Immunex Corp., USA
SO PCT Int. Appl., 45 pp.
CODEN: PIXXD2
PI WO 8804667 A1 880630
DS W: AU, DK, JP, KR
RW: AT, BE, CH, DE, FR, GB, IT, LU, NL, SE
AI WO 87-US3114 871204
PRAI US 86-944472 861219
US 87-119763 871112
DT Patent
LA English
AB Plasmids contg. interleukin-4 (IL-4) mutein-encoding DNA are prep'd. and the muteins are produced in and secreted from yeast. IL-4 or IL-4 muteins are useful for inducing proliferation and activation of antitumor or antiviral cytolytic T lymphocytes. Yeast expression plasmid pIXY157, contg. cDNA encoding a mating factor .alpha. leader peptide-human IL-4 mutein, i.e. GluAlaGluAla-[Asp-62, Asp-129]IL-4, under the control of the glucose-repressible ADH2 promoter, was constructed. Yeast transformed with this plasmid produced IL-4 mutein with 3.1 times. 107 units/mg activity (BCGF assay).

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File 155: MEDLINE(R) 1966-1998/Mar W3
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 (c) 1998 BIOSIS
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 (c) 1998 UMI
File 357: Derwent Biotechnology Abs 1982-1998/Feb B1
 (c) 1998 Derwent Publ Ltd
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 Royal Soc Chem & DECHEMA
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File 74: Int. Pharm. Abs. 1970-1997/Nov
 (c) 1997 Amer. Soc. of Health-System Pharm.

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Set	Items	Description
S1	34237	IL4 OR (INTERLEUKIN OR IL) (W) 4
S2	667627	MUTANT? OR MUTEIN? OR MUTAT?
S3	1881152	DERIV?
S4	1748987	REPLAC? OR SUBSTITUT? OR MODIF?
S5	1002504	AGONIST? OR ANTAGONIST?
S6	985352	TERMIN?
S7	76550	GLYCOSYLAT?
S8	24235	S1 NOT PY=(1996 OR 1997 OR 1998)
S9	6997	(POSITION? OR (AMINO(W)ACID?) OR RESIDUE?) (5N) (121 OR 124 - OR 125 OR 145 OR 148 OR 149)
S10	137874	S6(2N) (CARBOX? OR C)
S12	178670	S6(2N) (AMIN? OR N)
S17	16	(S2-S5) AND S8 AND S9 A
S23	2502	S1(10N) (S2-S5)
S25	13635	S12(8N) (S2 OR S4)
S26	7	S23 AND S25 AND S8 B
S27	14	S10(8N) (S2 OR S4) AND S23 AND S8 C
S28	13	S23 AND S8 AND S7 D

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 S29 7 RD S17 (unique items)

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29/7/1 (Item 1 from file: 155)
DIALOG(R) File 155: MEDLINE(R)
(c) format only 1998 Dialog Corporation. All rts. reserv.

08492829 96022606
A potent human ****interleukin****-****4**** ****antagonist****

stimulates the proliferation of murine cells expressing the human ****interleukin****-****4**** binding chain.

Davis ID; Treutlein HR; Friedrich K; Burgess AW

Ludwig Institute for Cancer Research, Melbourne Tumour Biology Branch,
Royal Melbourne Hospital, Victoria, Australia.

Growth Factors (SWITZERLAND) 1995, 12 (1) p69-83, ISSN 0897-7194

Journal Code: AOI

Languages: ENGLISH

Document type: JOURNAL ARTICLE

A single-amino-acid ****substitution**** ****mutant**** form of human ****interleukin****-****4**** (hIL-4), Y124D.hIL-4, has been described previously as an ****antagonist**** of the effects of hIL-4 on various human cells. The murine T-cell leukemic cell line CT.h4S, which expresses the human ****IL****-****4**** receptor, proliferates in response to both hIL-4 and murine ****IL****-****4****. Although Y124D.hIL-4 antagonizes the proliferative effects of hIL-4 on human phytohaemagglutinin-stimulated peripheral blood mononuclear cells, Y124D.hIL-4 is a potent stimulator for CT.h4S cells. Molecular modelling studies were performed to investigate the stability of different conformations of ****residue**** 124**** as well as the efficiency of different molecular mechanics force fields in homology modelling. We suggest that the aspartate ***substitution*** alters the C-terminal end of the D-helix in such way that the analogue still binds to the human ****IL****-****4**** receptor alpha-chain and signals through the murine gamma c-chain. In contrast, the Y124D.hIL-4/****IL****-****4**** receptor complex cannot signal through the human gamma c-chain.

29/7/2 (Item 2 from file: 155)

DIALOG(R) File 155: MEDLINE(R)

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08064898 95045516

Design of human ****interleukin****-****4**** ****antagonists**** inhibiting ****interleukin****-****4****-dependent and interleukin-13-dependent responses in T-cells and B-cells with high efficiency.

Tony HP; Shen BJ; Reusch P; Sebald W

Medizinische Poliklinik, Universitat, Wurzburg, Germany.

Eur J Biochem (GERMANY) Oct 15 1994, 225 (2) p659-65, ISSN 0014-2956

Journal Code: EMZ

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Human ****interleukin****-****4**** possesses two distinct sites for receptor activation. A signalling site, comprising residues near the C-terminus on helix D, determines the efficacy of ****interleukin****-****4**** signal transduction without affecting the binding to the ****interleukin****-****4**** receptor alpha subunit. A complete ****antagonist**** and a series of low-efficacy ****agonist**** variants of human ****interleukin****-****4**** could be generated by introducing combinations of two or three negatively charged aspartic acid residues in this site at ****positions**** 121****, 124****, and 125****. One of the double variants, designated [R121D,Y124D]****interleukin****-****4****, with ****replacements**** of both Arg121 and Tyr124 by aspartic acid residues was completely inactive in all analysed cellular responses. The loss of efficacy in [R121D,Y124D]****interleukin****-****4**** is estimated to be larger than 2000-fold. Variant [R121D,Y124D]****interleukin****-****4**** was also a perfect ****antagonist**** for inhibition of interleukin-13-dependent responses in B-cells and the TF-1 cell line with a Ki value of approximately 100 pM. In addition, inhibition of both ****interleukin****-****4**** -induced and interleukin-13-induced responses could be obtained by monoclonal antibody X2/45 raised against interleukin-4Rex, the extracellular domain of the ****interleukin****-****4**** receptor alpha subunit. These results indicate that efficient ****interleukin****-****4**** ****antagonists**** can be designed on the basis of a sequential two-step activation model. In addition, the experiments indicate the functional participation of the ****interleukin****-****4**** receptor alpha subunit in the interleukin-13

receptor system.

29/7/3 (Item 3 from file: 155)
DIALOG(R) File 155: MEDLINE(R)
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07892657 94202209

Aspects of receptor binding and signalling of ****interleukin****-****4**** investigated by site-directed mutagenesis and NMR spectroscopy.

Muller T; Dieckmann T; Sebald W; Oschkinat H
Theodor-Boveri-Institut fur Biowissenschaften (Biozentrum) Universitat
Wurzburg, Germany.

J Mol Biol (ENGLAND) Apr 8 1994, 237 (4) p423-36, ISSN 0022-2836

Journal Code: J6V

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Cytokines are hormones that carry information from cell to cell. This information is read from their surface upon binding to transmembrane receptors and by the subsequent initiation of receptor oligomerization. An influence on this process through mutagenesis on the hormone surface is highly desirable for medical reasons. However, an understanding of hormone-receptor interactions requires insight into the structural changes introduced by the ****mutations****. In this line structural studies on human ****IL****-****4**** and the medically important ****IL****-****4****

****antagonists**** Y124D and Y124G are presented. The site around Y124 is an important epitope responsible for the ability of ****IL****-****4**** to cause a signal in the target cells. It is shown that the local main-chain structure around ****residue**** ****124**** in the variants remains unchanged. A strategy is presented here which allows the study of these types of proteins and their variants by NMR which does not require carbon labelled samples.

29/7/4 (Item 4 from file: 155)

DIALOG(R) File 155: MEDLINE(R)
(c) format only 1998 Dialog Corporation. All rts. reserv.

07829886 94065589

An ****interleukin****-****4**** (****IL****-****4****) ****mutant**** protein inhibits both ****IL****-****4**** or IL-13-induced human immunoglobulin G4 (IgG4) and IgE synthesis and B cell proliferation: support for a common component shared by ****IL****-****4**** and IL-13 receptors.

Aversa G; Punnonen J; Cocks BG; de Waal Malefyt R; Vega F Jr; Zurawski SM ; Zurawski G; de Vries JE

Human Immunology Department, DNAX Research Institute, Palo Alto, California 94304-1104.

J Exp Med (UNITED STATES) Dec 1 1993, 178 (6) p2213-8, ISSN 0022-1007

Journal Code: I2V

Languages: ENGLISH

Document type: JOURNAL ARTICLE

****Interleukin****-****4**** (****IL****-****4****) and IL-13 share many biological functions. Both cytokines promote growth of activated human B cells and induce naive human surface immunoglobulin D+ (sIgD+) B cells to produce IgG4 and IgE. Here we show that a ****mutant**** form of human ****IL****-****4****, in which the tyrosine ****residue**** at ****position**** ****124**** is ****replaced**** by aspartic acid (hIL-4.Y124D), specifically blocks ****IL****-****4**** and IL-13-induced proliferation of B cells costimulated by anti-CD40 mAbs in a dose-dependent fashion. A mouse ****mutant****-****IL****-****4**** protein (mIL-4.Y119D), which antagonizes the biological activity of mouse ****IL****-****4****, was ineffective. In addition, hIL-4.Y124D, at concentrations of up to 40 nM, did not affect IL-2-induced B cell proliferation. hIL-4.Y124D did not have detectable ****agonistic**** activity in these B cell proliferation assays. Interestingly, hIL-4.Y124D also strongly inhibited both ****IL****-

****4**** or IL-13-induced IgG4 and IgE synthesis in cultures of peripheral blood mononuclear cells, or highly purified sIgD+ B cells cultured in the presence of anti-CD40 mAbs. ****IL****-****4**** and IL-13-induced IgE responses were inhibited > 95% at a approximately 50- or approximately 20-fold excess of hIL-4.Y124D, respectively, despite the fact that the ****IL****-****4**** mutant protein had a weak ****agonistic**** activity. This ****agonistic**** activity was 1.6 +/- 1.9% (n = 4) of the maximal IgE responses induced by saturating concentrations of ****IL****-****4****. Taken together, these data indicate that there are commonalities between the ****IL****-****4**** and IL-13 receptor. In addition, since hIL-4.Y124D inhibited both ****IL****-****4**** and IL-13-induced IgE synthesis, it is likely that ****antagonistic**** mutant**** ****IL****-****4**** proteins may have potential clinical use in the treatment of IgE-mediated allergic diseases.

29/7/5 (Item 5 from file: 155)

DIALOG(R) File 155: MEDLINE(R)

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07717024 94085387

Two distinct functional sites of human ****interleukin**** ****4**** are identified by variants impaired in either receptor binding or receptor activation.

Kruse N; Shen BJ; Arnold S; Tony HP; Muller T; Sebald W
Theodor-Boveri-Institut fur Biowissenschaften (Biozentrum) der
Universitat, Wurzburg, Germany.

EMBO J (ENGLAND) Dec 15 1993, 12 (13) p5121-9, ISSN 0261-4189

Journal Code: EMB

Languages: ENGLISH

Document type: JOURNAL ARTICLE

****Interleukin**** ****4**** (****IL****-****4****) exerts a decisive role in the coordination of protective immune responses against parasites, particularly helminths. A disregulation of ****IL****-****4**** function is possibly involved in the genesis of allergic disease states. The search for important amino acid residues in human ****IL****-****4**** by ****mutational**** analysis of charged invariant amino acid positions identified two distinct functional sites in the 4-helix-bundle protein. Site 1 was marked by amino acid ****substitutions**** of the glutamic acid at position 9 in helix A and arginine at position 88 in helix C. Exchanges at both positions led to ****IL****-****4**** variants deficient in binding to the extracellular domain of the ****IL****-****4**** receptor (IL-4R(ex)). In parallel, up to 1000-fold increased concentrations of this type of variant were required to induce T-cell proliferation and B-cell CD23 expression. Site 2 was marked by amino acid exchanges in helix D at ****positions**** ****121****, ****124**** and ****125**** (arginine, tyrosine and serine respectively in the wild-type). ****IL****-****4**** variants affected at site 2 exhibited partial ****agonist**** activity during T-cell proliferation; however, they still bound with high affinity to IL-4R(ex). [The generation of an ****IL****-****4**** ****antagonist**** by ****replacing**** tyrosine 124 with aspartic acid has been described before by Kruse et al. (1992) (EMBO J., 11, 3237-3244)]. These findings indicate that ****IL****-****4**** functions by binding IL-4R(ex) via site 1 which is constituted by residues on helices A and C. (ABSTRACT TRUNCATED AT 250 WORDS)

29/7/6 (Item 6 from file: 155)

DIALOG(R) File 155: MEDLINE(R)

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05303152 87149079

Molecular cloning and expression of a human B-cell growth factor gene in Escherichia coli [see comments]

Sharma S; Mehta S; Morgan J; Maizel A

Science (UNITED STATES) Mar 20 1987, 235 (4795) p1489-92, ISSN

0036-8075 Journal Code: UJ7

Contract/Grant No.: 16672; CA38499, CA, NCI; CA39798, CA, NCI

Comment in Science 1994 Aug 19;265(5175):1110-1

Languages: ENGLISH

Document type: JOURNAL ARTICLE

A human B-cell growth factor (BCGF) (12 kilodaltons) supports the clonal proliferation of B lymphocytes. A clone was isolated that contained the proper structural sequence to encode biologically active, 12-kilodalton BCGF in Escherichia coli and to hybridize to a specific messenger RNA, identified by in vitro translation in Xenopus laevis oocytes. A relatively hydrophobic region of 18 amino acids was found at the amino terminal of the ****124****-****amino**** ****acid**** -long polypeptide. The carboxyl terminal is composed of at least 32 amino acids that are ****derived**** from nucleotide sequences bearing significant homology to the Alu repeat family.

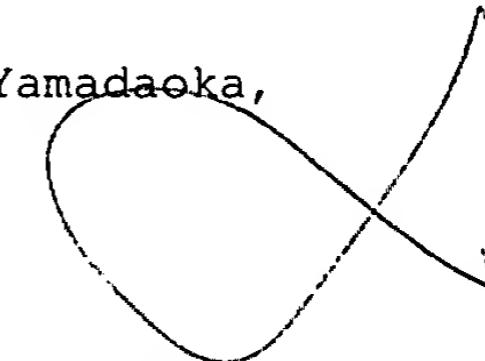
29/7/7 (Item 1 from file: 5)
DIALOG(R) File 5:BIOSIS PREVIEWS(R)
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11636688 BIOSIS Number: 98236688

Mapping of murine Th1 and Th2 helper T-cell epitopes on fimbriae from Porphyromonas gingivalis

Ogawa T; Uchida H; Yasuda K

Dep. Oral Microbiology, Osaka Univ. Faculty Dentistry, Yamadaoka,
Suita-Osaka 565, Japan



Journal of Medical Microbiology 42 (3). 1995. 165-170.

Full Journal Title: Journal of Medical Microbiology

ISSN: 0022-2615

Language: ENGLISH

Print Number: Biological Abstracts Vol. 099 Iss. 011 Ref. 158864

Th1- and Th2-****derived**** cytokine production in response to synthetic peptides of the fimbrial subunit protein (fimbrilin) from Porphyromonas gingivalis strain 381 was assessed in spleen mononuclear cells (MNC) of BALB/c mice (H-2-d haplotype) immunised with the fimbrial protein antigen and adjuvant GM-53 in Freund's incomplete adjuvant (FIA). Sixty-seven sequential overlapping 10-mer peptides covering the complete 337 amino-acids (AA) protein of P. gingivalis fimbrilin were synthesised. Stimulation of spleen MNC in vitro with these 10-mer peptides resulted in the production of murine interleukin-2 (IL-2), gamma-interferon (IFN-gamma), ****IL****-****4****, IL-5 and IL-6. Peptides 13 (AA 61-70), 24 (AA 116-125), 31 (AA 151-160) and 64 (AA 316-325) markedly induced IL-2 production. In particular, peptide 24 (DPLKIKRVHA), which contained I-A-d, I-E-d and I-A-k binding motifs, was the most potent stimulator of IL-2, IFN-gamma, ****IL****-****4****, IL-5 and IL-6 production. Spleen MNC from C3H/HeN mice (H-2-k) followed by BALB/c mice (H-2-d) immunised with peptide 24 were high responders to peptide 24 in terms of both IFN-gamma and ****IL****-****4**** production, whereas A/J mice (H-2-a) and C57BL/6 mice (H-2-b) were very low responders. P. gingivalis fimbriae evoked higher delayed-type hypersensitivity (DTH) reactions in B10.D2 (H-2-d) and B10.BR (H-2-k) mice followed by C57BL/10 (B10, H-2-b) and B10.A (H-2-a) and in guinea-pigs immunised with the fimbriae and GM-53 in FIA. Thus, the Th1 and Th2 helper T cell cytokine-producing responses and the DTH reactions to P. gingivalis fimbriae in mice are restricted by H-2 haplotype and the ****amino****-****acid**** sequence (AA 116-****125****) within the fimbrial protein molecule acts as a common stimulator of cytokine production in both Th1 and Th2 cells.

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S30 7 S26 NOT

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S31 3 RD (unique items)

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31/7/1 (Item 1 from file: 155)

DIALOG(R)File 155: MEDLINE(R)

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08549184 96172541

Circularly permuted ****interleukin**** ****4**** retains proliferative and binding activity.

Kreitman RJ; Puri RK; McPhie P; Pastan I

Laboratory of Molecular Biology, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892, USA.

Cytokine (UNITED STATES) May 1995, 7 (4) p311-8, ISSN 1043-4666

Journal Code: A52

Languages: ENGLISH

Document type: JOURNAL ARTICLE

In human ****interleukin**** ****4****(****IL****-****4****), the carboxyl and amino termini of the 129 amino acid hormone are close to each other and this region is believed to be important for binding to the ****IL****-****4**** receptor (IL-4r). We constructed plasmids encoding circularly permuted ****IL****-****4**** ****mutants**** with the peptide Gly-Gly-Asn-Gly-Gly (GGNGG) joining the carboxyl to the ****amino**** terminus and with new ****amino**** and carboxyl ****termini**** elsewhere. ****Mutant**** ****IL****-****4****(38-37) is composed of ****IL****-****4**** residues 38-129 GGNNG and 1-37. ****Mutant**** ****IL****-****4****(105-104) is composed of ****IL****-****4**** residues 105-129, GGNNG and 1-104. ****IL****-****4****(38-37) and ****IL****-****4****(105-104) were purified from E. coli to near homogeneity and retained 50-100% of the binding and proliferative activity of ****IL****-****4****, and in addition retained the ability to upregulate CD23 on Burkitt's lymphoma cells. Circular dichroism studies indicated that the tertiary structures of both ****IL****-****4****(38-37) and ****IL****-****4****(105-104) were retained, with the former molecule most similar to native ****IL****-****4****. We conclude that while both native termini of ****IL****-****4**** may be near its binding site, neither is required to be free for optimum activity.

31/7/2 (Item 2 from file: 155)

DIALOG(R)File 155: MEDLINE(R)

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08316690 95339319

Increased antitumor activity of a circularly permuted ****interleukin**** ****4****-toxin in mice with ****interleukin**** ****4**** receptor-bearing human carcinoma.

Kreitman RJ; Puri RK; Pastan I

Laboratory of Molecular Biology, National Cancer Institute, NIH, Bethesda, Maryland 20892, USA.

Cancer Res (UNITED STATES) Aug 1 1995, 55 (15) p3357-63, ISSN 0008-5472 Journal Code: CNF

Languages: ENGLISH

Document type: JOURNAL ARTICLE

We reported previously that circularly permuted ****interleukin****-****4**** (****IL4****), composed of amino acids 38-129 of ****IL4****

connected by a linker peptide GGNNGG to amino acids 1-37, is preferable to native ****IL4**** for fusing to the amino terminus of truncated *Pseudomonas* exotoxin (PE) to make a recombinant toxin, because the new ligand-toxin junction results in improved ****IL4**** receptor (IL4R)-binding (R. J. Kreitman et al., Proc. Natl. Acad. Sci. USA, 91: 6889-6893, 1994). We now report that the improved binding of circularly permuted ****IL4****-toxin is associated with improved antitumor activity in tumor-bearing mice. For *in vivo* testing, we made an improved circularly permuted ****IL4****-toxin, termed ****IL4****(38-37)-PE38KDEL. It contains an N38D ****mutation**** at the ****amino**** ****terminus****, allowing improved expression and large-scale production in *Escherichia coli*. It also contains the truncated toxin PE38KDEL, which is composed of amino acids 253-364 and 381-608 of PE, followed by KDEL. To evaluate antitumor activity, nude mice carrying s.c. tumors composed of IL4R-bearing human A431 epidermoid carcinoma cells were injected with recombinant toxins i.v. every other day for three doses. ****IL4****(38-37)-PE38KDEL induced complete remissions in 80% of mice receiving 50 micrograms/kg x 3 and 100% of mice receiving 100 micrograms/kg x 3, while only 70% of mice receiving 200 micrograms/kg x 3 of the native ****IL4****-toxin ****IL4****-PE38KDEL obtained complete remission. Disease-free survival after obtaining complete remissions was higher in mice treated with ****IL4****(38-37)-PE38KDEL 50 micrograms/Kg QOD x 3 than with ****IL4****-PE38KDEL 200 micrograms/Kg QOD x 3 ($P < 0.03$). ****IL4****(38-37)-PE38KDEL and ****IL4****-PE38KDEL exhibited similar toxicity and pharmacokinetics in the mice, indicating that the improved antitumor activity of the circularly permuted ****IL4****-toxin was due to its improved binding to the IL4R on the target cells.

31/7/3 (Item 1 from file: 73)
DIALOG(R) File 73:EMBASE
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8573785 EMBASE No: 92249712

A receptor binding domain of mouse ****interleukin****-****4**** defined by a solid-phase binding assay and *in vitro* mutagenesis

Morrison B.W.; Leder P.

Department of Genetics, Howard Hughes Medical Institute, Harvard Medical School, Boston, MA 02115 USA

J. BIOL. CHEM. (USA), 1992, 267/17 (11957-11963) CODEN: JBCHA ISSN: 0021-9258

LANGUAGES: English SUMMARY LANGUAGES: English

****Interleukin**** ****4**** (****IL****-****4****) is a potent, pleiotropic lymphokine that affects a variety of cells, especially those of hematopoietic origin. Although murine and human ****IL****-****4**** are homologous proteins, they display a species specificity in which murine ****IL****-****4**** acts only upon mouse cells, and human ****IL****-****4**** only upon human cells. We have used a mutagenesis strategy to define both the structural determinants of this specificity and a receptor binding domain of murine ****IL****-****4****. To do this, we developed convenient solid-phase binding assays for mouse and for human ****IL****-****4****, each utilizing receptor-immunoglobulin fusion proteins and alkaline phosphatase-tagged ligands. These were employed to assess the receptor binding activities of wild type and ****mutant**** forms of ****IL****-****4****. In a separate biological assay, we measured the ability of each version of ****IL****-****4**** to induce proliferation of a cultured mouse T-cell line. By ****replacing**** regions of mouse ****IL****-****4**** with homologous segments of human ****IL****-****4****, we found that the amino-terminal 16 residues and the carboxyl-terminal 20 residues of murine ****IL****-****4**** are required for species-specific receptor binding as well as for T-cell proliferation. A major portion of the amino acid sequence between these regions can be substituted between mouse and human without loss of receptor binding or biological activity. Further, alanine-scanning mutagenesis revealed specific residues in the amino- and carboxyl-terminal regions (Glu-12, Ile-14, Leu-104, Asp-106, Phe-107, and Leu-111) that bear side chains critical for function. An analysis of the carboxyl-terminal region of

murine ****IL****-****4**** and its comparison with carboxyl-terminal regions of other related cytokines suggest an evolutionary conservation of structural and functional features.

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14 S27
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S32 9 S27 NOT (S17 OR S26)

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S33 5 RD (unique items)

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33/7/1 (Item 1 from file: 155)

DIALOG(R) File 155: MEDLINE(R)

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08228268 95238326

Species-specific ****agonist****/****antagonist**** activities of human ****interleukin****-****4**** variants suggest distinct ligand binding properties of human and murine common receptor gamma chain.

Bonsch D; Kammer W; Lischke A; Friedrich K

Theodor-Boveri-Institut fur Biowissenschaften (Biozentrum), Universitat Wurzburg, Federal Republic of Germany.

J Biol Chem (UNITED STATES) Apr 14 1995, 270 (15) p8452-7, ISSN 0021-9258 Journal Code: HIV

Languages: ENGLISH

Document type: JOURNAL ARTICLE

****Interleukin****-****4**** (****IL****-****4****) is a pleiotropic cytokine eliciting various responses in target cells upon binding to its receptor. Activation of the ****IL****-****4**** receptor probably involves interaction of the ligand with both the ****IL****-****4**** receptor alpha subunit (IL-4R alpha) and the common gamma chain (c gamma). Although human and murine ****IL****-****4**** receptor alpha chains are specific for ****IL****-****4**** from the same species, murine c gamma can form a signal-competent complex with human IL-4R alpha (hIL-4R alpha) and human ****IL****-****4**** (hIL-4). We have generated a hIL-4 responsive murine myeloid cell line (FDC-4G) expressing a chimera comprising the extracellular domain of human IL-4R alpha and the intracellular domain of human granulocyte colony-stimulating factor receptor (hG-CSFR). This hybrid receptor was shown to form a complex with hIL-4 and the murine c gamma-chain. Biological activities of human ****IL****-****4**** variants on murine FDC-4G cells and on the human erythroleukemic cell line TF-1 displayed a strikingly different pattern. Single amino acid ****replacements**** at two different positions in the ****C****-****terminal**** helix of hIL-4, the region of the previously defined "signaling site," lead to an inverse agonist/antagonist behavior of the resulting cytokines in the two cellular systems. From these findings we conclude that upon formation of the activated ****IL****-****4**** receptor complex murine and human c gamma interact with hIL-4 in a geometrically different fashion.

33/7/2 (Item 2 from file: 155)

DIALOG(R) File 155: MEDLINE(R)

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08029300 95001869

Site-specific conjugation to ****interleukin**** ****4**** containing ****mutated**** cysteine residues produces ****interleukin**** ****4**** -toxin conjugates with improved binding and activity.

Kreitman RJ; Puri RK; Leland P; Lee B; Pastan I

Laboratory of Molecular Biology, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892.

Biochemistry (UNITED STATES) Sep 27 1994, 33 (38) p11637-44, ISSN 0006-2960 Journal Code: A0G

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Fusion of a ligand to another protein frequently impairs the binding of the ligand. Recombinant toxins composed of ****mutants**** of *Pseudomonas* exotoxin (PE) fused to the ****C*****-****terminus**** of human ****interleukin**** ****4**** (****IL4****) are cytotoxic to ****IL4**** receptor- (IL4R-) bearing tumor cells but bind to the IL4R with only 1% the affinity of ****IL4****. We have developed a method to connect a toxin to a ligand which allows the junction to be moved to a location on the ligand which would minimize the binding impairment. We designed ****mutants**** of ****IL4**** in which residue 28, 38, 68, 70, 97, or 105 was substituted with cysteine. All purified mutants bound to the IL4R with 60-100% the affinity of ****IL4****, indicating that the ****IL4**** structure was essentially unchanged. The ****IL4**** ****mutants**** were then each conjugated through a disulfide bond to PE35, a truncated form of PE which contains a single cysteine. ****IL4**** conjugated to PE35 at residue 28, 38, or 105 of ****IL4**** bound with 10-fold improved affinity and was 10-fold more cytotoxic than the recombinant ****IL4****-toxin in which PE is fused to position 129 at the C-terminus of ****IL4****. ****IL4**** containing PE35 conjugated at position 68, 70, or 97 had lower binding affinity and cytotoxic activity. These results indicate that the location of the ligand-protein junction can be selectively moved to enhance conjugate effectiveness, and implications could be made regarding which regions of ****IL4**** are important for binding.

33/7/3 (Item 1 from file: 73)

DIALOG(R) File 73:EMBASE

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9681448 EMBASE No: 95235845

A potent human ****interleukin****-****4**** ****antagonist**** stimulates the proliferation of murine cells expressing the human ****interleukin****-****4**** binding chain

Davis I.D.; Treutlein H.R.; Friedrich K.; Burgess A.W.

Ludwig Institute for Cancer Research, Melbourne Tumour Biology Branch, PO Royal Melbourne Hospital, Melbourne, Vic. 3050 Australia

Growth Factors (United Kingdom) , 1995, 12/1 (69-83) CODEN: GRFAE

ISSN: 0897-7194

LANGUAGES: English SUMMARY LANGUAGES: English

33/7/4 (Item 2 from file: 73)

DIALOG(R) File 73:EMBASE

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9547349 EMBASE No: 95116961

Distinct sequence motifs within the cytoplasmic domain of the human ****IL****-****4**** receptor differentially regulate apoptosis inhibition and cell growth

Deutsch H.H.J.; Koetttnitz K.; Chung J.; Kalthoff F.S.

Abteilung Immunregulation (MRG-IR), Sandoz Forschungsinstitut, Brunner Strasse 59, A-1235 Vienna Austria

Journal of Immunology (USA) , 1995, 154/8 (3696-3703) CODEN: JOIMA

ISSN: 0022-1767

LANGUAGES: English SUMMARY LANGUAGES: English

Hematopoietin receptors generally function as multimeric complexes composed of a unique ligand-binding chain and a second component often shared between several members of this receptor family. To better understand the signal transduction of the human ****IL*****-****4**** receptor (hIL4R), we analyzed the functionality of targeted ****mutations**** in two cytoplasmic regions of the ligand-binding hIL4R chain that we previously identified to be necessary for growth mediation in factor-dependent murine Ba/F3 cells. Here, we provide evidence that transient inhibition of apoptotic death of Ba/F3 cells and the competence to proliferate indefinitely depend on separated and distinct sequence motifs of the hIL4R. In particular, hIL4R constructs with a truncation of the recently described gp130 box1 from P242 to K264, or a deletion of the acidic region between S330 and S365, fail to stimulate growth or to mediate the inhibition of apoptosis. hIL4R bearing a point mutation within the gp130 box1 (P242S) is defective for growth stimulation but still signals the transient inhibition of apoptotic cell death and the induction of c-myc RNA. A third region required for ****IL4****-mediated cell growth is localized between T462 and S476 and includes the sequence NPAY previously described to serve as interaction motif in signaling of epidermal growth factor and insulin receptors. Conversion of Y472 into F472 within the latter hIL4R motif affects the competence of stably transfected BA/F3 cells to proliferate indefinitely in the presence of hIL4. Sequences C-terminal of S476 are not essential for growth stimulation of BA/F3 transfectants.

33/7/5 (Item 3 from file: 73)
DIALOG(R) File 73:EMBASE
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9546712 EMBASE No: 95117963

Importance of the glutamate residue of KDEL in increasing the cytotoxicity of *Pseudomonas* exotoxin derivatives and for increased binding to the KDEL receptor

Kreitman R.J.; Pastan I.
Laboratory of Molecular Biology, National Cancer Institute, National Institutes of Health, 9000 Rockville Pike, Bethesda, MD 20892 USA
Biochemical Journal (United Kingdom) , 1995, 307/1 (29-37) CODEN: BIJOA
ISSN: 0264-6021

LANGUAGES: English SUMMARY LANGUAGES: English

It was previously shown that amino acids 609-613 (REDLK) at the C-terminus of *Pseudomonas* exotoxin (PE) are necessary for cytotoxicity, presumably by directing the toxin to the endoplasmic reticulum (ER). Using the anti-(interleukin 2 receptor (IL2R)) immunotoxin anti-Tac(Fv)-PE38 (AT-PE38REDLK), it was found that removing the terminal lysine did not alter the activity, but replacing REDL with KDEL, the most common ER retention sequence, increased activity. To determine which amino acid in KDEL was responsible for the increase in activity, we tested eight ****C*****-****terminal**** ****mutants**** of AT-PE38REDLK. Using IL2R-bearing MT-1 cells, we found that the glutamate residue of KDEL was required for high activity, as the cytotoxicity of AT-PE38 ending in KDEL, RDEL, KEEL or REEL was much greater than that of AT-PE38 ending in REDL, KEDL, RDDL or KDDL. Using freshly isolated lymphocytic leukaemia cells, AT-PE38 ending in KDEL, REEL or RDEL was more than 100-fold more cytotoxic than AT-PE38 ending in KEDL, REDL, RDDL or the native sequence REDLK. The RDEL sequence also improved the cytotoxic activity of an ****interleukin**** ****4****-PE38 toxin fusion protein. Improved cytotoxic activity correlated with improved binding of the C-termini to the KDEL receptor on rat Golgi membranes. These data indicate that the glutamate residue of KDEL improves the cytotoxicity of PE by increasing binding to a sorting receptor which transports the toxin from the transreticular Golgi apparatus to the ER, where it is translocated to the cytosol and inhibits protein synthesis.

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S34 13 S28 NOT (S17 OR S26 OR S27)

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S35 8 RD (unique items)

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35/7/1 (Item 1 from file: 155)

DIALOG(R) File 155: MEDLINE(R)

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08213779 95221405

High level expression and refolding of mouse ****interleukin****
****4**** synthesized in Escherichia coli.

Levine AD; Rangwala SH; Horn NA; Peel MA; Matthews BK; Leimgruber RM;
Manning JA; Bishop BF; Ollins PO

Searle Discovery Research, Monsanto, St. Louis, Missouri 63198, USA.

J Biol Chem (UNITED STATES) Mar 31 1995, 270 (13) p7445-52, ISSN

0021-9258 Journal Code: HIV

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Mouse ****Interleukin**** ****4**** is a 20-kDa glycoprotein, synthesized by activated T lymphocytes and mast cells, which regulates the growth and/or differentiation of a broad spectrum of target cells of the immune system, including B and T lymphocytes, macrophages, and hematopoietic progenitor cells. Using an inducible recA promoter and the g10-L ribosome-binding site, recombinant non-****glycosylated**** ****interleukin**** ****4**** (****IL****-****4****) was expressed as 17% of total cellular protein in Escherichia coli inclusion bodies, as a reduced, inactive 14.5-kDa polypeptide. The protein was refolded and aggregates dissociated when three disulfide bonds were reformed by slowly decreasing the concentration of guanidine hydrochloride and cysteine. The oxidized monomer was purified to homogeneity by sequential ion-exchange and size exclusion chromatography. When compared with native ****IL****-****4****, E. coli-****derived**** ****IL****-****4**** displayed an identical specific activity of 4-7 x 10(7) units/mg. This recombinant ****IL****-****4**** contained a three-amino-acid NH2-terminal extension, which did not affect its biological activity. Purified biologically active protein consisted of three isoforms as shown by two-dimensional gel electrophoresis, with a pI greater than 9.0. These data suggest that neither ****glycosylation**** nor the NH2 terminus of mouse ****IL****-****4**** play a critical role in contributing to its in vitro biological activity.

35/7/2 (Item 2 from file: 155)

DIALOG(R) File 155: MEDLINE(R)

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08184277 95186667

Murine ****interleukin****-****4**** production with baculovirus: an easy and rapid method for a small scale production of functional interleukins.

Cottrez F; Auriault C; Capron A; Kusznier JP; Groux H

Unité CNRS-URA 1854, Institut Pasteur de Lille, France.

Eur Cytokine Netw (FRANCE) Sep-Oct 1994, 5 (5) p481-7, ISSN 1148-5493

Journal Code: A56

Languages: ENGLISH

Document type: JOURNAL ARTICLE

We described a baculovirus expression system for high level production of secreted murine recombinant ****IL****-****4****. We have constructed a recombinant baculovirus based on *Autographa californica* polyhedrosis virus, containing both a synthetic PCR-****derived**** murine ****IL****-****4**** cDNA under the control of the polyhedrin promoter and the lacZ gene under the control of the P10 promoter to allow an easy detection of recombinant virus. The baculovirus ****IL****-****4**** was fully functional in biological assay and was present under two ****glycosylated**** forms in the supernatants of infected Sf9 cells. We also detected a third unglycosylated intracytoplasmic form resulting from a fusion between the 35 first amino acids of polyhedrin and the murine ****IL****-****4****. Finally, confocal microscopy showed that this recombinant protein was secreted along a classical pathway like in mammalian cells.

35/7/3 (Item 1 from file: 5)
DIALOG(R) File 5: BIOSIS PREVIEWS(R)
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5647101 BIOSIS Number: 33042122
CHARACTERIZATION OF RECOMBINANT HUMAN ****INTERLEUKIN**** ****4****
****DERIVED**** FROM COS-7 MONKEY KIDNEY CELLS
LE H V; LABDON J E; RAMANATHAN L; MAYS C; SYTO R; NAGABHUSHAN T L; TROTTA
P P
SCHERING CORP., BLOOMFIELD, N.J. 07003.
78TH ANNUAL MEETING OF THE AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS,
PHILADELPHIA, PENNSYLVANIA, USA, JUNE 7-11, 1987. FED PROC 46 (6). 1987.
2230. CODEN: FEPRA
Language: ENGLISH

35/7/4 (Item 1 from file: 357)
DIALOG(R) File 357: Derwent Biotechnology Abs
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178302 DBA Accession No.: 95-05123 PATENT
Modified ****unglycosylated**** hematopoietic growth factor, e.g.
granulocyte-macrophage colony stimulating factor - granulocyte or
granulocyte-macrophage colony stimulating factor or interleukin protein
engineering for hematopoietic growth factor-antagonist activity
AUTHOR: Vadas M A; Lopez A F; Shannon M F
PATENT ASSIGNEE: Medvet-Sci. 1995
PATENT NUMBER: WO 9504075 PATENT DATE: 950209 WPI ACCESSION NO.:
95-090611 (9512)
PRIORITY APPLIC. NO.: AU 944772 APPLIC. DATE: 940330
NATIONAL APPLIC. NO.: WO 94AU432 APPLIC. DATE: 940728
LANGUAGE: English
ABSTRACT: A new modified hematopoietic growth factor (HGF) is
****unglycosylated****, and has 1 or more exposed acidic amino acids
(aa, e.g. Glu and/or Asp) in the 1st alpha-helix substituted with a
basic aa residue (e.g. Arg and/or Lys). The HGF may be human or animal
granulocyte-macrophage colony stimulating factor (GM-CSF, preferred),
interleukin-2, interleukin-3, ****interleukin****-****4****,
interleukin-5, interleukin-6, interleukin-7, interleukin-9,
interleukin-10, granulocyte colony stimulating factor or
erythropoietin. The modified HGF acts as an HGF-antagonist, e.g. in
ameliorating the aberrant effects of an endogenous HGF. In an example,
wild-type GM-CSF was expressed in *Escherichia coli*, using plasmid
pshGM-CSF containing a human GM-CSF cDNA artificial gene cloned in
plasmid pIN-III-OmpH3. GME21R was expressed from plasmid pSGM21.1,
containing Glu-21 to Arg substitution. GME21K was expressed from
plasmid pSGM21.4, containing a Glu-21 to Lys substitution. The
recombinant proteins were expressed in *E. coli* MC1061 (wild-type) or *E.*
coli BL21 (mutants) after induction by IPTG. (51pp)

35/7/5 (Item 2 from file: 357)
DIALOG(R) File 357:Derwent Biotechnology Abs
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120017 DBA Accession No.: 91-07659

Biochemical characterization of recombinant extracellular domain of the human ****IL****-****4**** receptor and generation of monoclonal antibodies - human recombinant soluble ****interleukin****-****4**** receptor extracellular domain gene cloning and expression in COS-7 cell culture; monoclonal antibody production (conference abstract)

AUTHOR: Galizzi J P; Djossou O; Garrone P; Ait-Yahia S; Banchereau J

CORPORATE AFFILIATE: Schering-Plough

CORPORATE SOURCE: Schering-Plough, Laboratory for Immunological Research, BP 11, 69571 Dardilly Cedex, France.

JOURNAL: J.Cell.Biochem. (Suppl.15F, 111) 1991

CODEN: JCEBD5

LANGUAGE: English

ABSTRACT: cDNA encoding the 130 kDa human ****interleukin****-****4**** receptor (****IL****-****4****-R) has been cloned. The cDNA encodes an open reading frame of 825 amino acid residues including an extracellular domain of 207 amino acids with 6 N-linked potential ****glycosylation**** sites, a single transmembrane domain of 24 amino acids and an intracellular domain of 569 amino acids. 2 cDNAs encoding the extracellular domain of the ****IL****-****4****-R were constructed using 2 different endonuclease restriction sites (DraIII and AhaII). Proteins secreted by transformed COS-7 cells blocked ****IL****-****4**** binding to cells. The affinity purified soluble ****IL****-****4****-R (DraIII construct) bound ****IL****-****4**** with high affinity since the Rd value (50-100 pM) was close to the Kd for ****IL****-****4**** binding to the membrane receptor. The purified soluble ****IL****-****4****-R secreted by COS-7 cells was a glycoprotein of 45,000 Da including 25,000 Da for the oligosaccharide moiety. Treatment of soluble ****IL****-****4****-R with N-glycanase or endoglycosidase-F did not affect ****IL****-****4**** binding to the receptor. ****IL****-****4****-R was a powerful ****antagonist**** of ****IL****-****4**** in vitro (binding 1:1). Purified soluble ****IL****-****4****-R was used to produce monoclonal antibodies against ****IL****-****4****-R. (0 ref)

35/7/6 (Item 3 from file: 357)
DIALOG(R) File 357:Derwent Biotechnology Abs
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078541 DBA Accession No.: 88-09390 PATENT

Human ****interleukin****-****4**** analogs - used for inducing proliferation and activation of antitumor or virucide cytolytic I lymphocytes

PATENT ASSIGNEE: Immunex 1988

PATENT NUMBER: WO 8804667 PATENT DATE: 880630 WPI ACCESSION NO.:
88-190617 (8827)

PRIORITY APPLIC. NO.: US 119763 APPLIC. DATE: 871112

NATIONAL APPLIC. NO.: WO 87US3114 APPLIC. DATE: 871204

LANGUAGE: English

ABSTRACT: A new human ****interleukin****-****4**** (hIL-4) analog protein comprises at least 1 amino acid ****substitution****, deletion, or insertion resulting in inactivation of asparagine-linked ****glycosylation**** site, deletion or substitution of a Cys residue, or a modification of a yeast KEX2 protease recognition site. Also new are: (1) a pharmaceutical composition for induction of proliferation and lytic activity in antitumor cytolytic T-lymphocytes which comprises the hIL-4 analog and a suitable carrier; (2) a DNA sequence encoding hIL-4 analog protein; (3) a recombinant expression vector containing the new DNA sequence; (4) a method for preparing an hIL-4 analog protein comprising culturing a microorganism transformed with a recombinant expression vector; (5) an antiviral composition; and (6) an

antitumor composition. The analogs have a mutant amino acid sequence which is homologous to the wild-type hIL-4 sequence except that at least 1 Asn-A'-Z in the wild-type is replaced by Asn-A2-Y or X-A2-A3, where A1, A2, A3 = any amino acid; X = any amino acid not Asn; Y = any amino acid not Z; Z = Ser or Thr. The alterations may be by site-directed mutagenesis, etc. (52pp)

35/7/7 (Item 1 from file: 358)
DIALOG(R) File 358: Current BioTech Abs
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027769 CBA Acc. No.: 06-10-004499 DOC. TYPE: Patent
Human ****interleukin****-****4**** ****muteins****.
AUTHOR: Anderson, D. M.; Cosman, D. J.; Deeley, M. C.; Grabstein, K. H.;
Price, V. L.
CODEN: P1XXD2
PATENT NUMBER: WO 8804667
PATENT APPLICATION: US 944472 (861219)
COMPANY: Immunex, USA
PUBLICATION DATE: 30 Jun 1988 (880630) LANGUAGE: English
ABSTRACT: Recombinant biologically active human ****interleukin****-****4**** ****mutant**** analogue proteins are described in which the N-linked ****glycosylation**** sites have been inactivated. The muteins were produced in yeast expression vectors.

35/7/8 (Item 1 from file: 73)
DIALOG(R) File 73: EMBASE
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9824511 EMBASE No: 95373357
Structural analysis of the CHO-****derived**** ****interleukin****-****4**** by liquid-chromatography/electrospray ionization mass spectrometry
Tsarbopoulos A.; Pramanik B.N.; Nagabhushan T.L.; Covey T.R.
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Journal of Mass Spectrometry (United Kingdom) , 1995, 30/12 (1752-1763)
CODEN: JMSPF ISSN: 1076-5174
LANGUAGES: English SUMMARY LANGUAGES: English
Electrospray (ES) ionization mass spectrometric analysis of CHO-****derived**** recombinant ****interleukin****-****4**** (****IL****-****4****) before and after deglycosylation with peptide: N-glycosidase F is described. That proved useful not only in deriving the size of the attached carbohydrate components but also in identifying the major glycoforms as the mono-and disialylated complex-type N-linked oligosaccharides. Additional signals arising from glycoforms containing carbohydrate components with more extended or higher branching were also detected. Further mapping of CHO ****IL****-****4**** was carried out by combining proteolytic digestion and chromatographic separation of the resulting peptide mixture with on-line ES mass spectrometric detection, Comparative analysis of the V8 protease digests of CHO ****IL****-****4**** and its deglycosylated product revealed the Asn38 N-****glycosylation**** site. Glycopeptide-containing fractions were identified by searching the resulting raw ES data for signal pairs whose m/z values differ by the mass of various carbohydrate units adjusted for the signal's charge state (e.g. 97 u difference for a triply charged ES signal of a glycopeptide containing NeuAc units). Furthermore, ES mass spectrometric analysis at elevated orifice potentials allowed the rapid location of the glycopeptides in the total ion current chromatogram by monitoring several carbohydrate-specific fragment ions. This high orifice-induced fragmentation is a highly sensitive method for generating sugar diagnostic ions in chromatographically separated components, even when glycopeptide and peptide fragments are co-eluting.
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